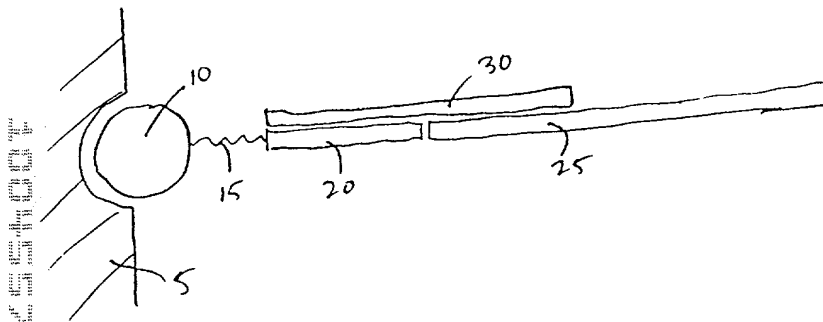
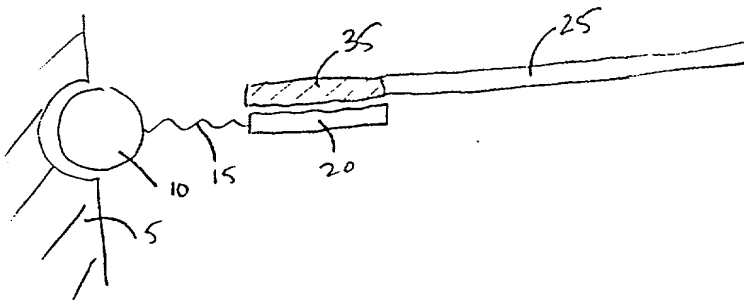


A



B



C

Fig 1

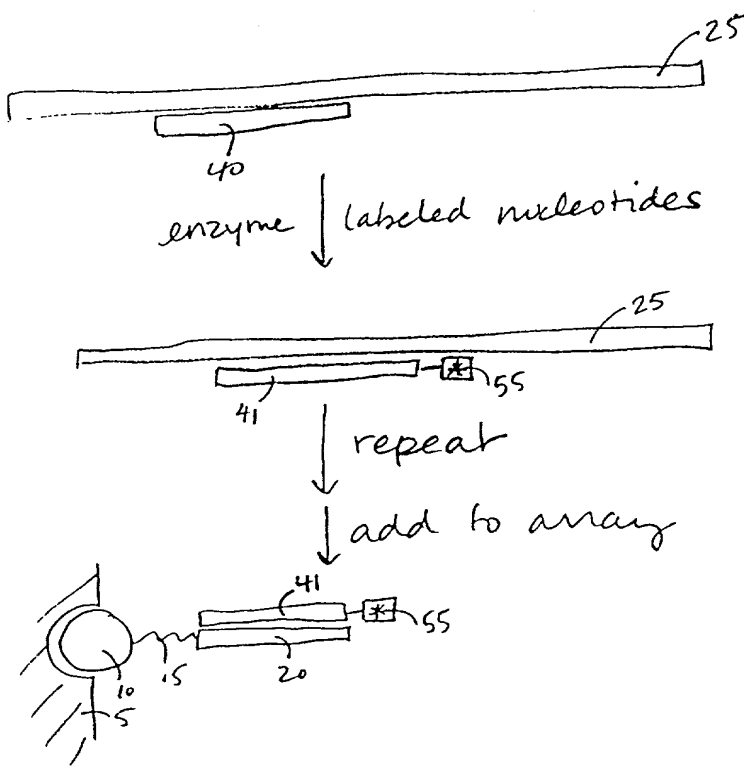


Fig 2A

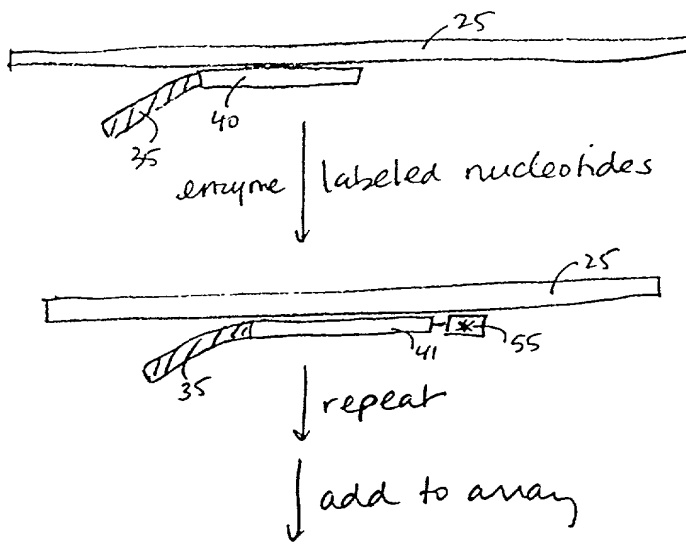


Fig 2B

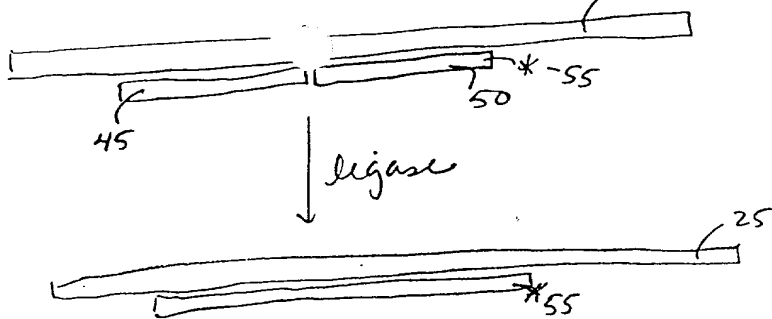
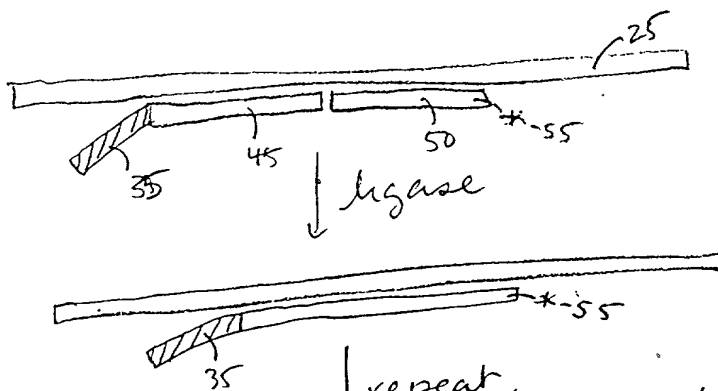
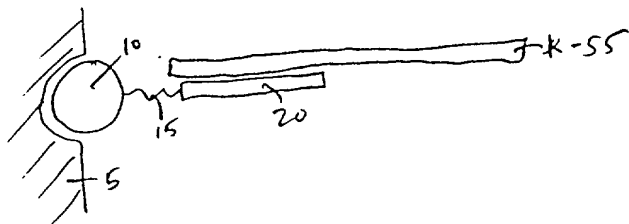


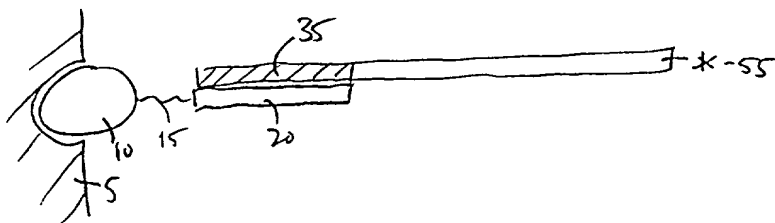
Fig 3A

repeat,  
remove unligated probes as needed



repeat,  
remove unligated probes as needed

Fig 3B



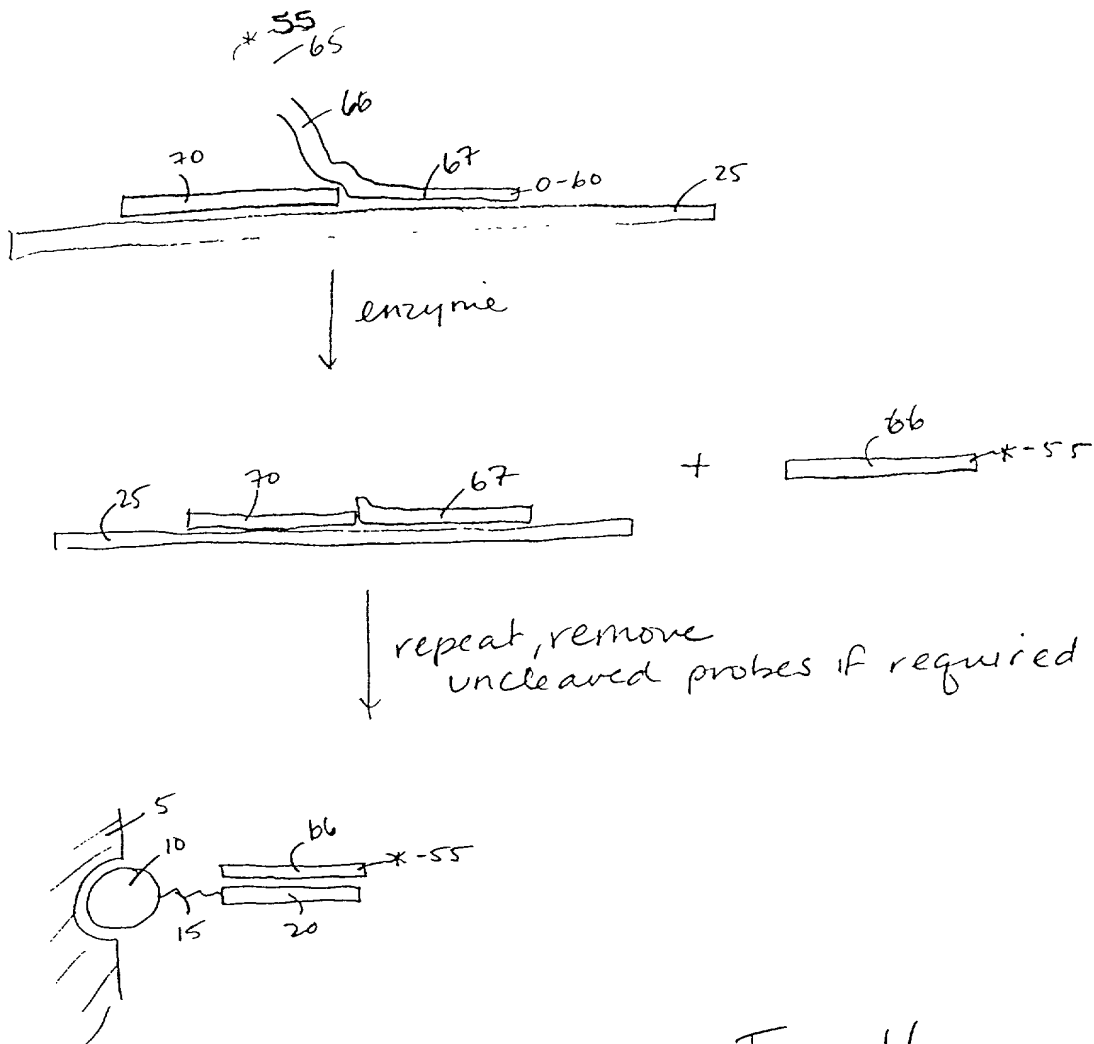


Fig 4

FIG 5A

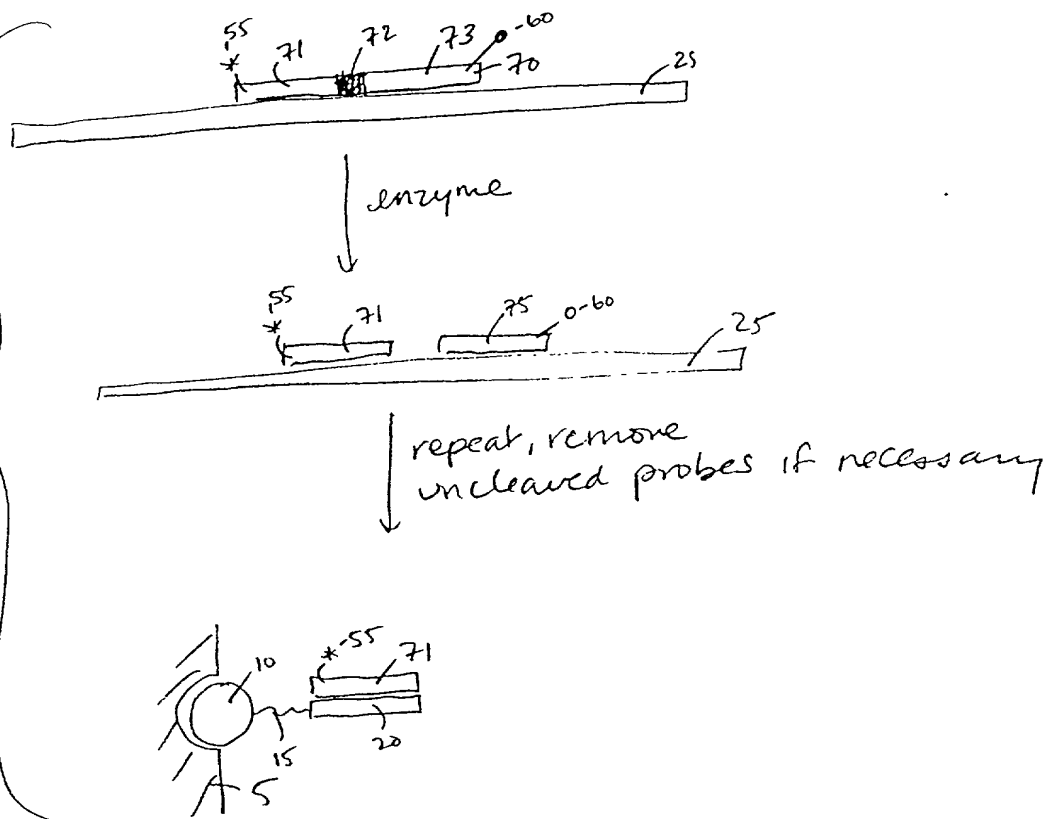
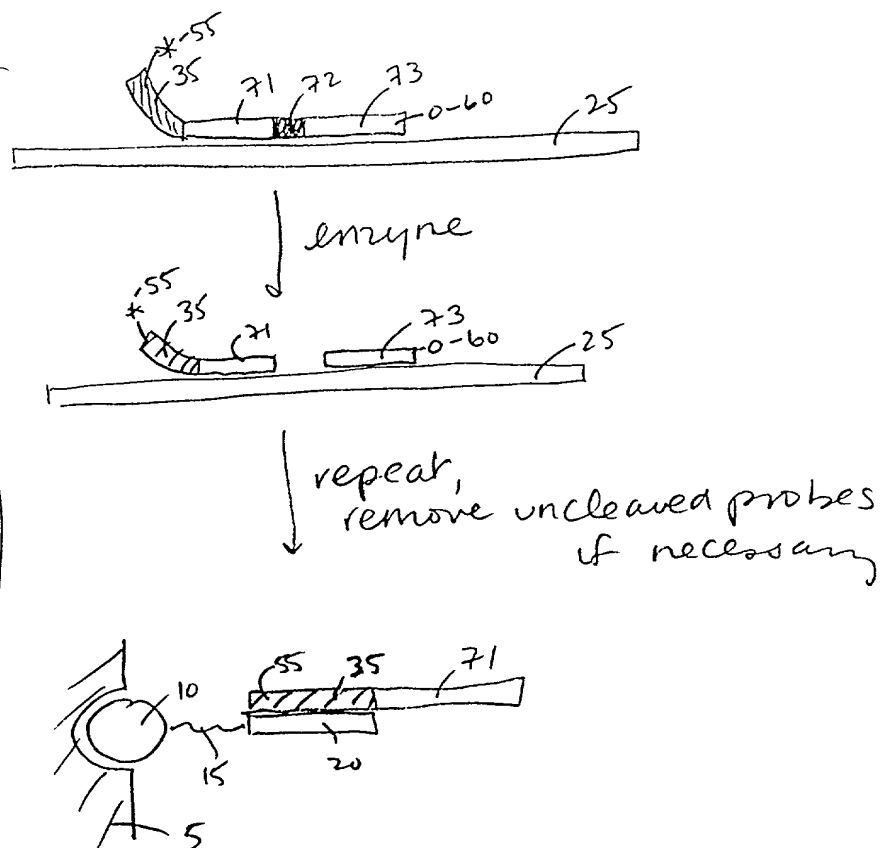


FIG 5B



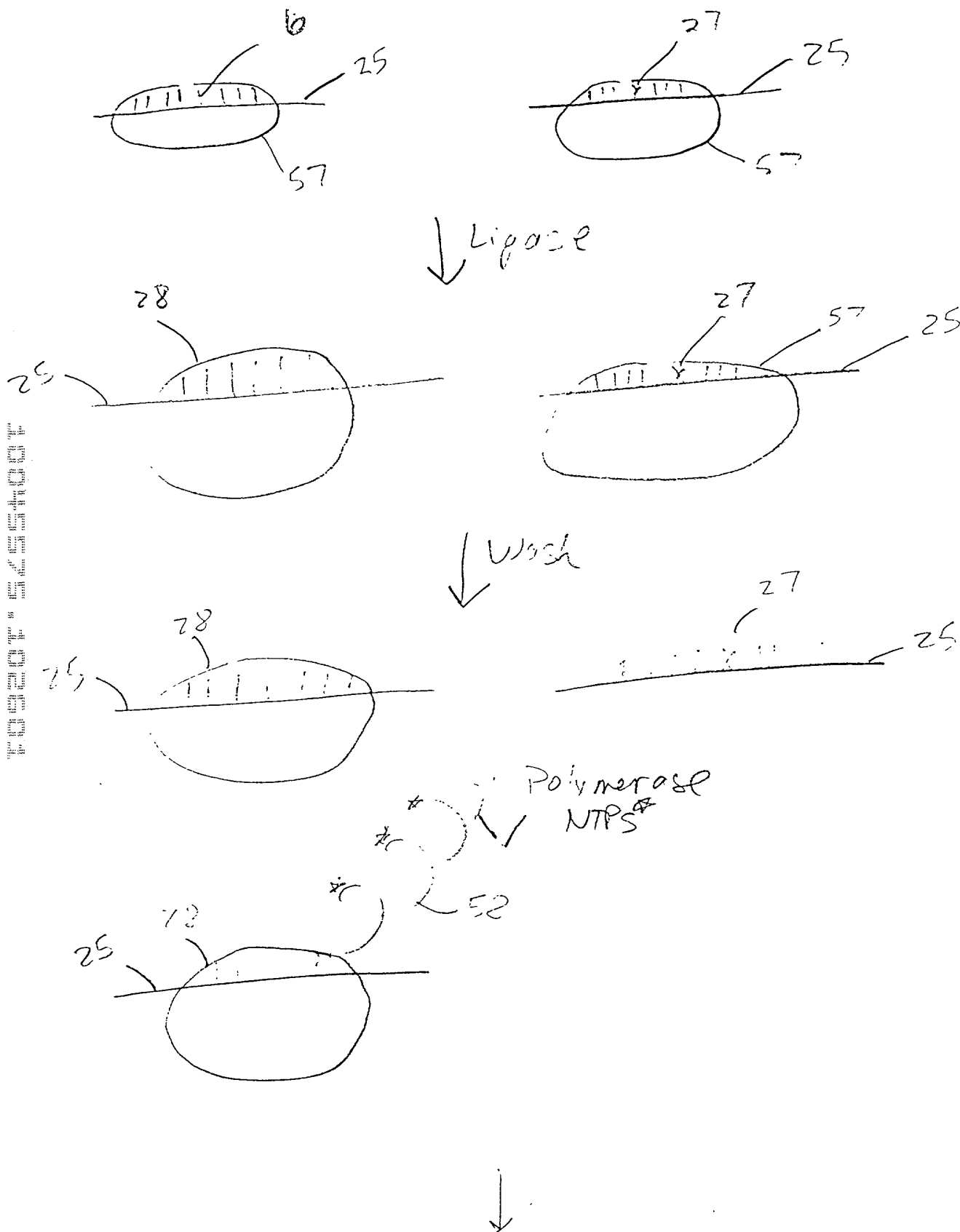


FIGURE 6

↓ Restriction  
Endonuclease

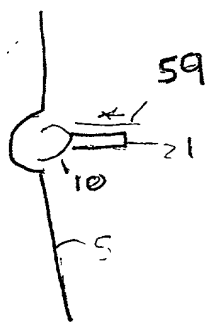
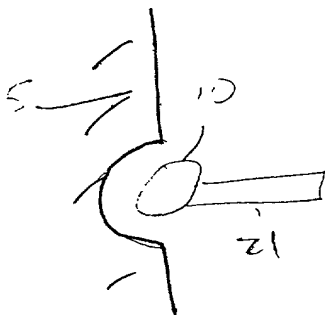
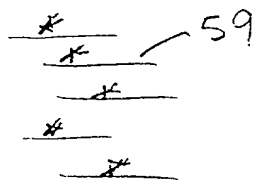
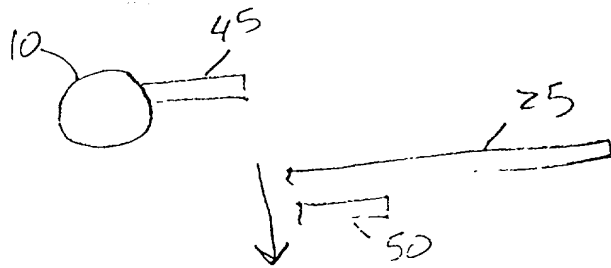
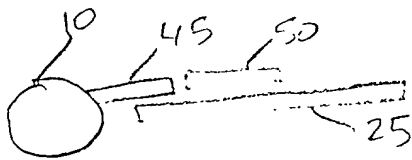


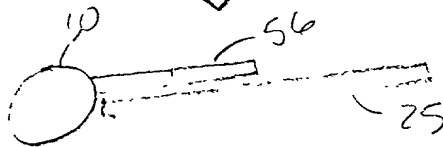
FIGURE 6  
Continued



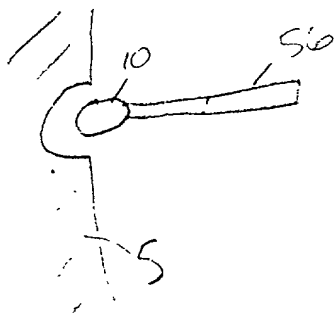
A



DLA



Derivative  
Array



RCA primer

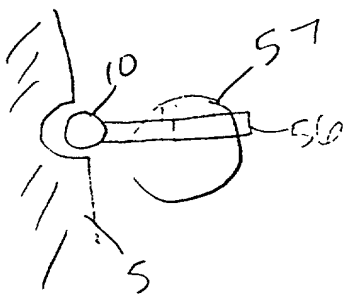
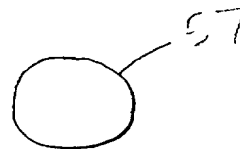


FIGURE 7



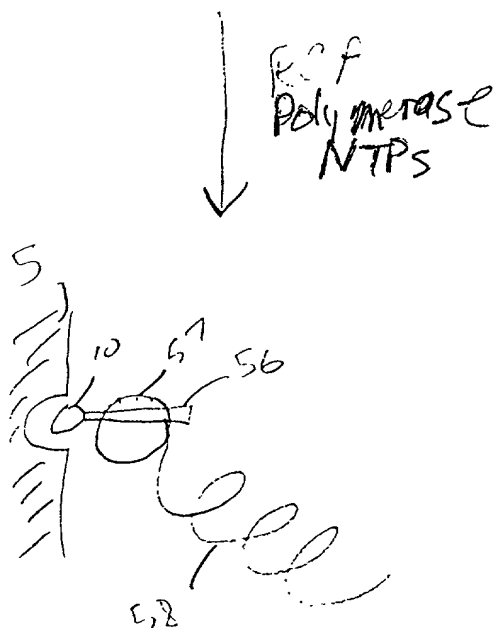


FIGURE 7  
(continued)

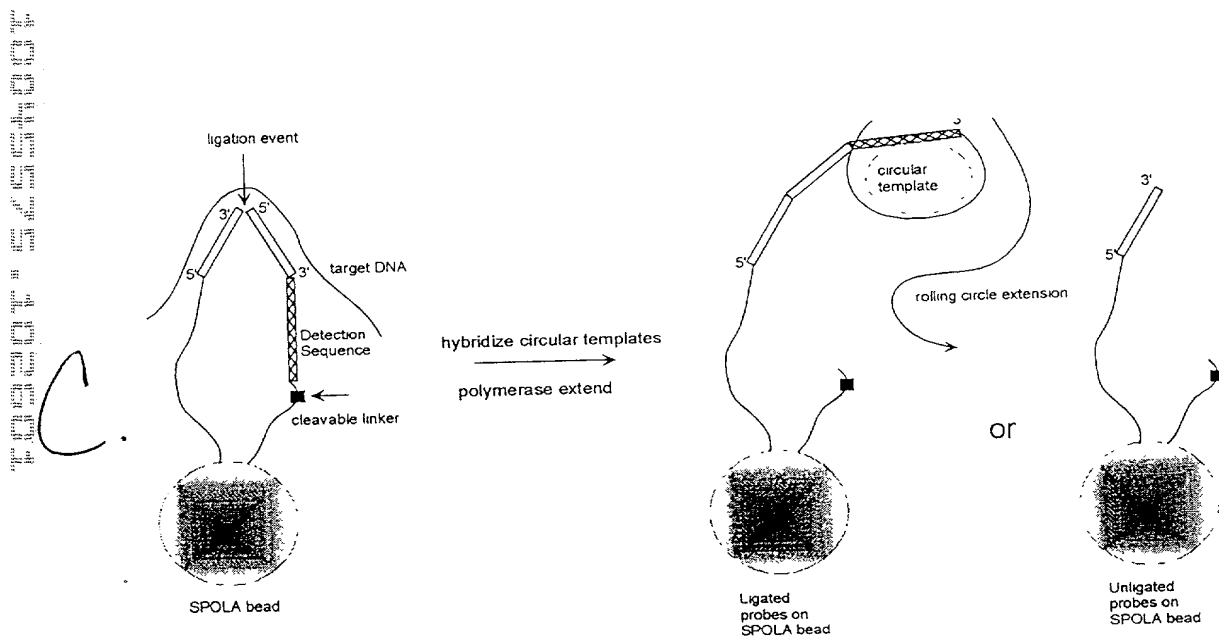
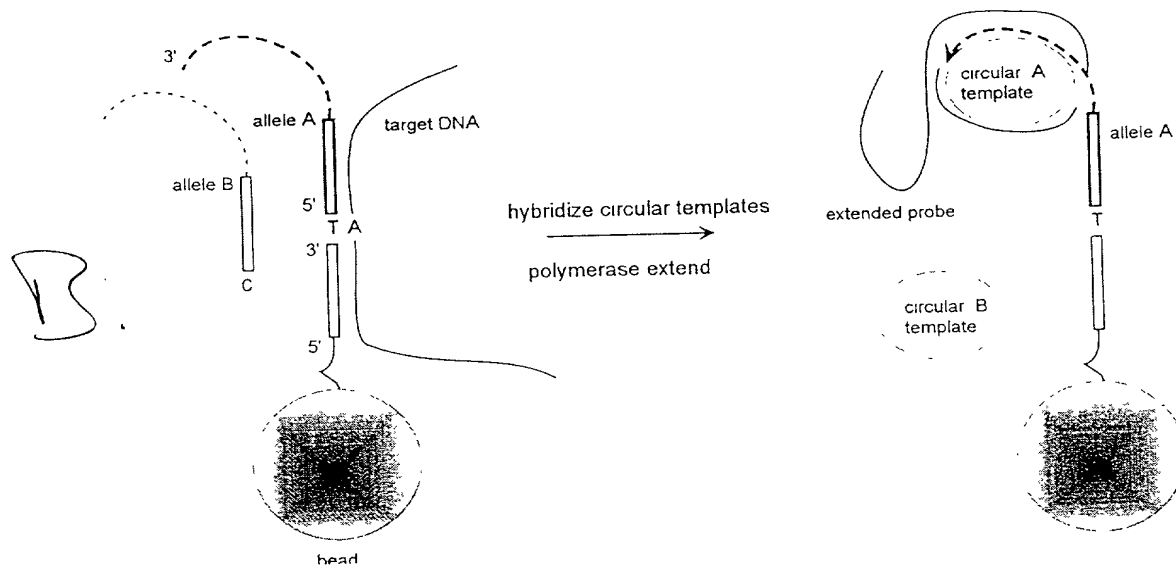
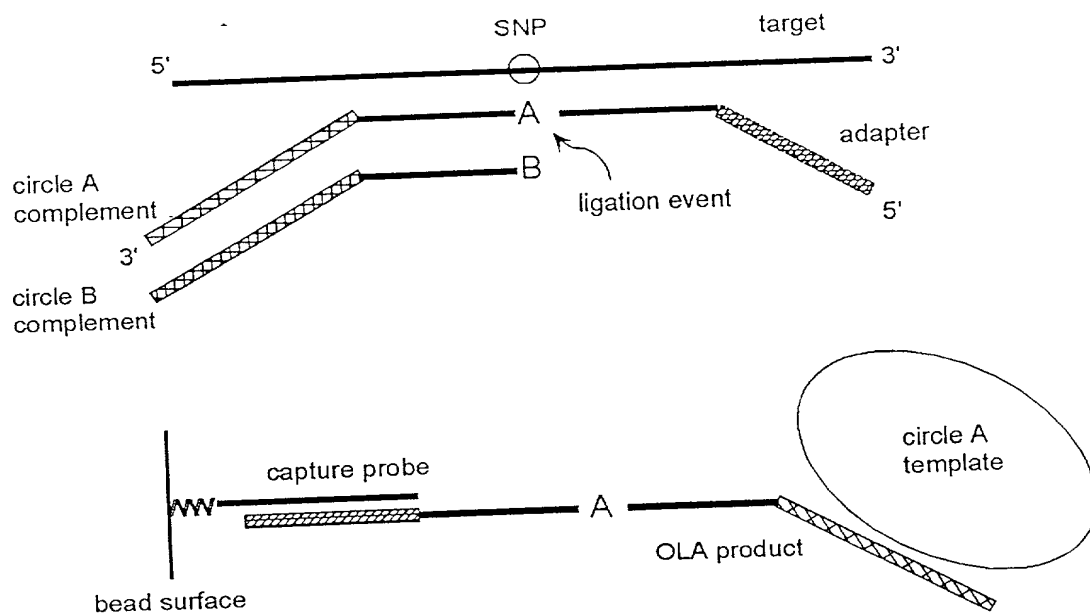
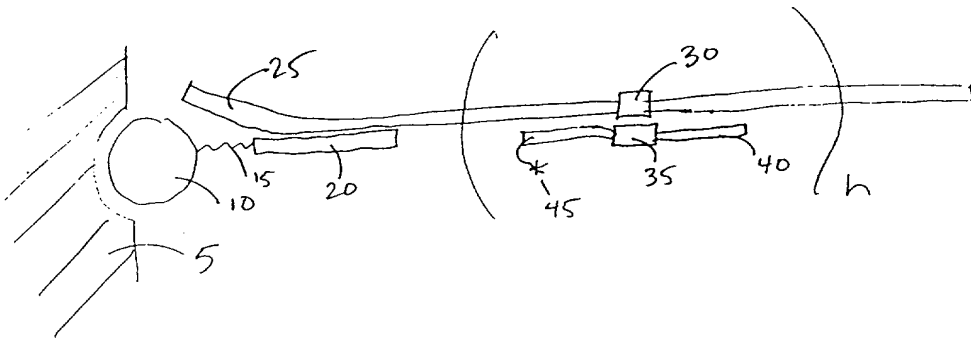


Figure 7 continued

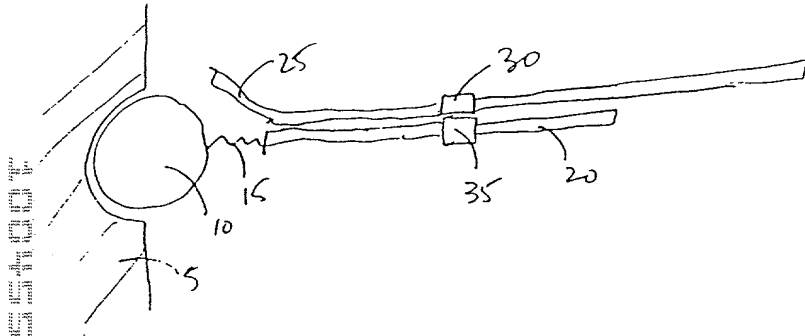


D

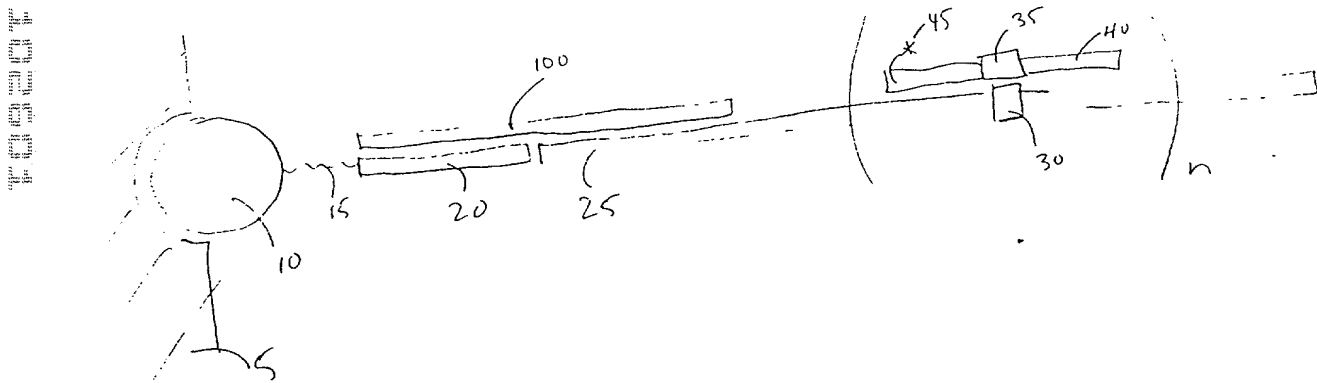
Figure 7 continued



A

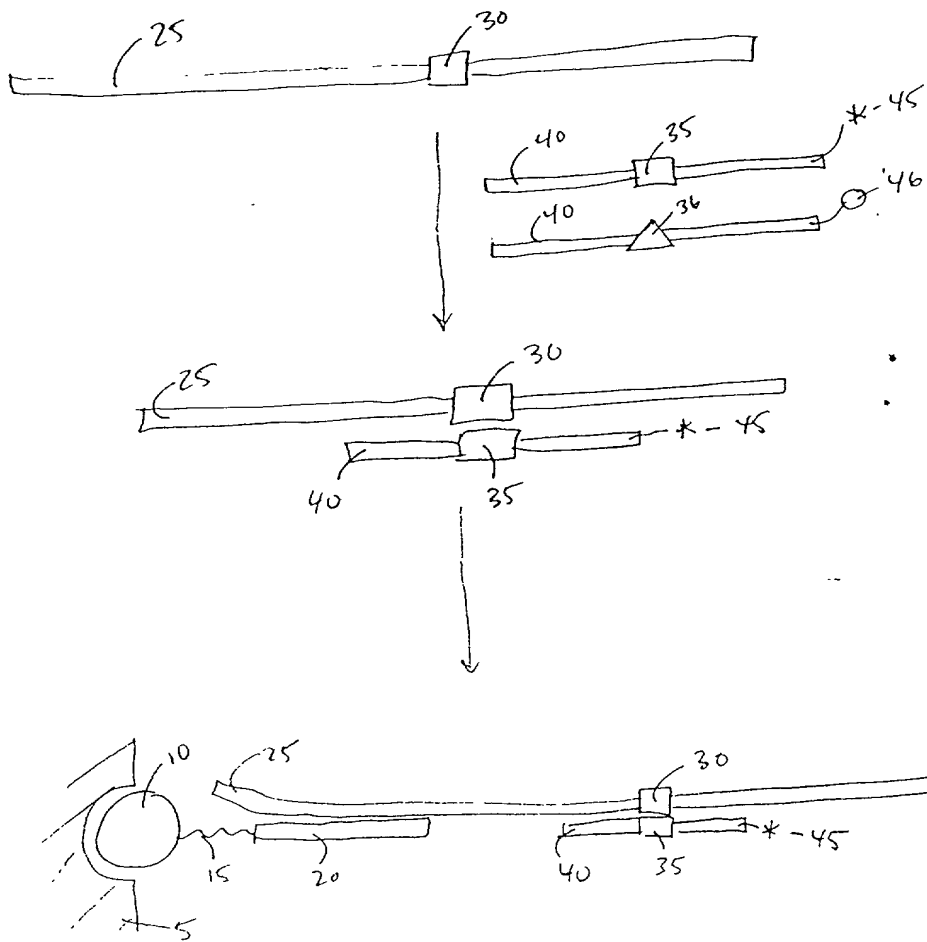


B

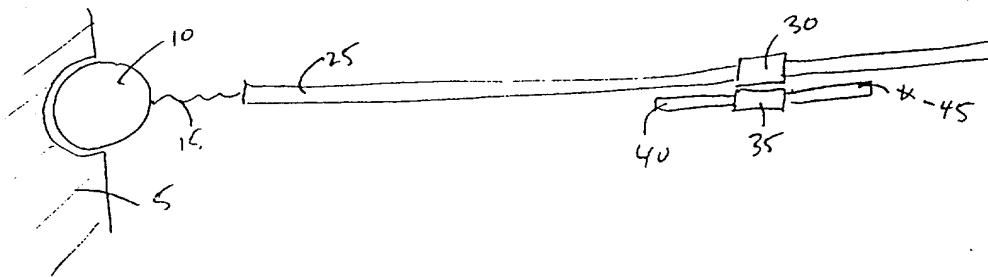


C

FIGURE 8

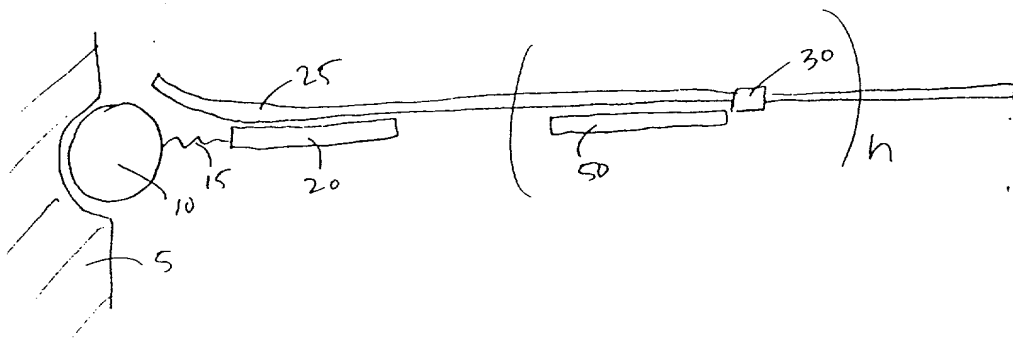


D

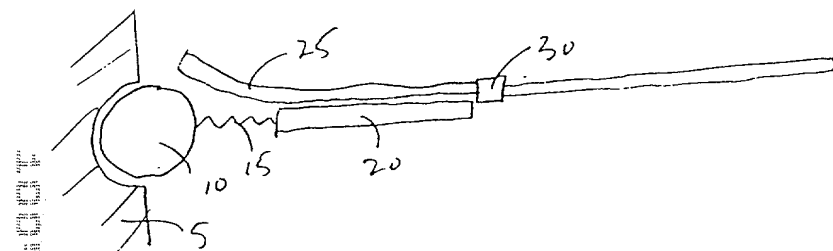


E

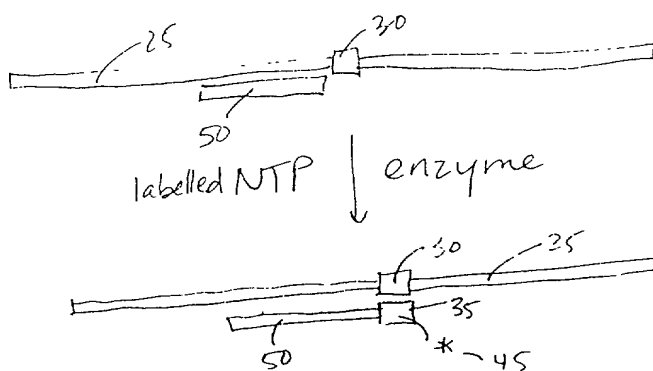
FIGURE 8  
(continued)



A



B



labelled NTP

enzyme

optional  
removal of  
unextended  
primers

denature,  
add to array

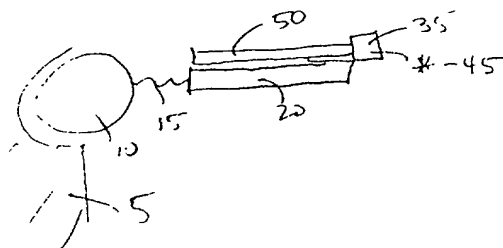


FIGURE 9.

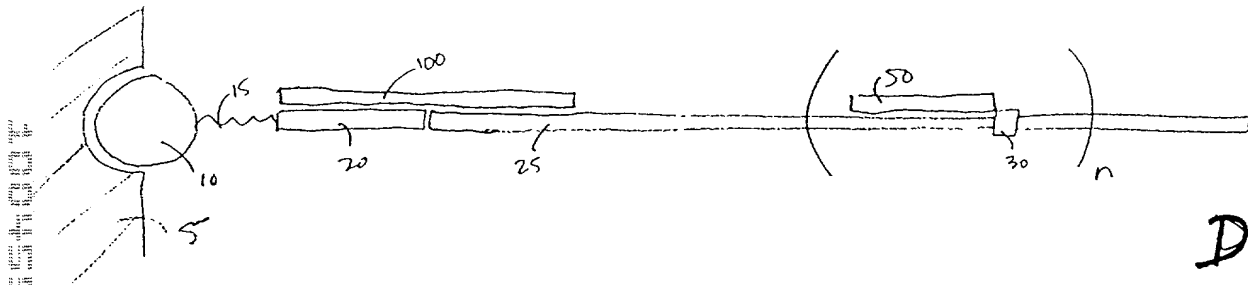
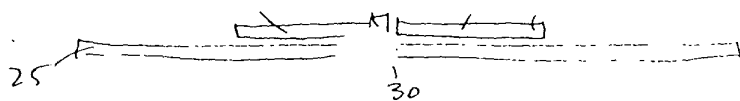
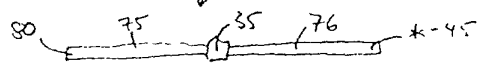


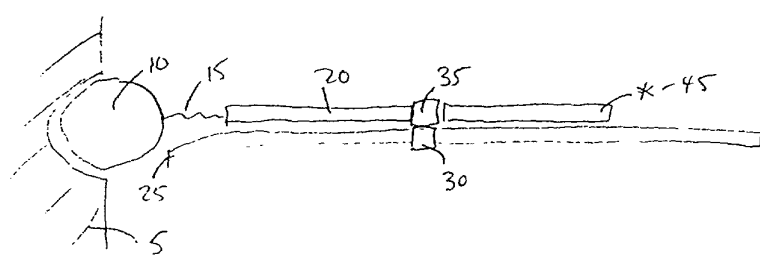
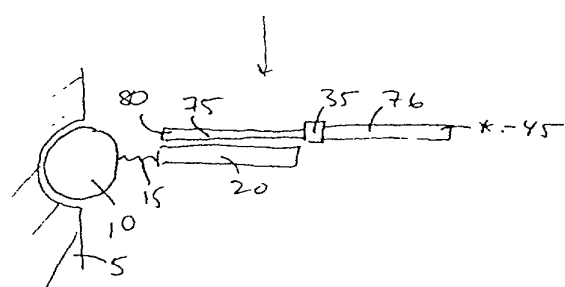
FIGURE 9  
(continued)



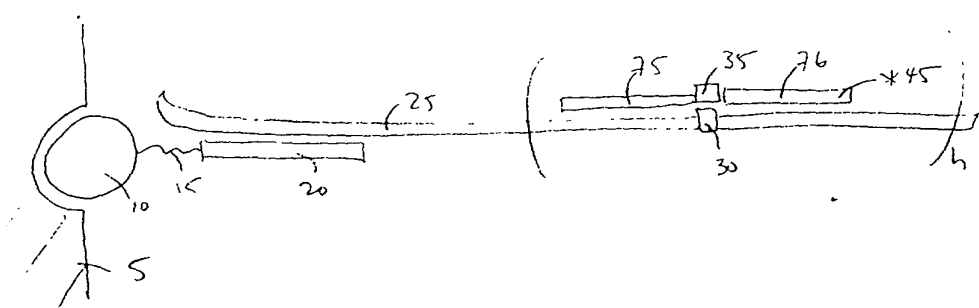
ligase



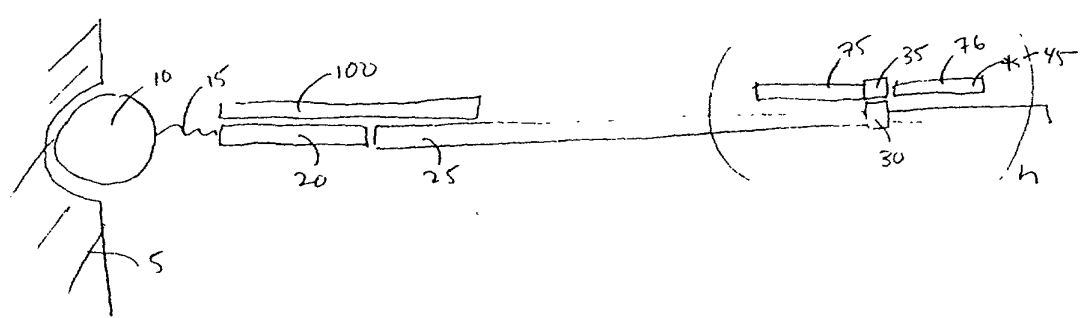
A



B



C

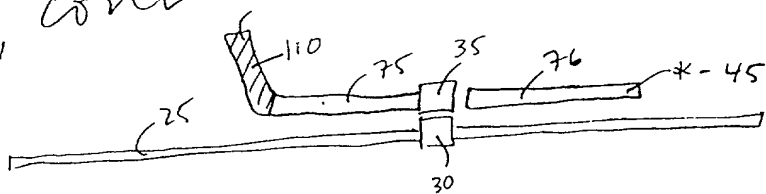


D

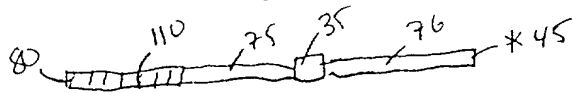
FIGURE 10



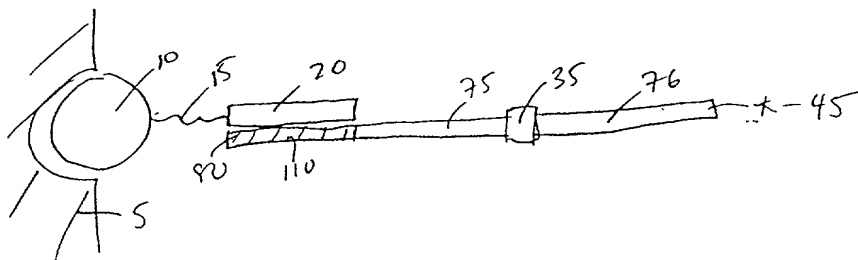
3, cont



↓  
ligate



↓  
to array

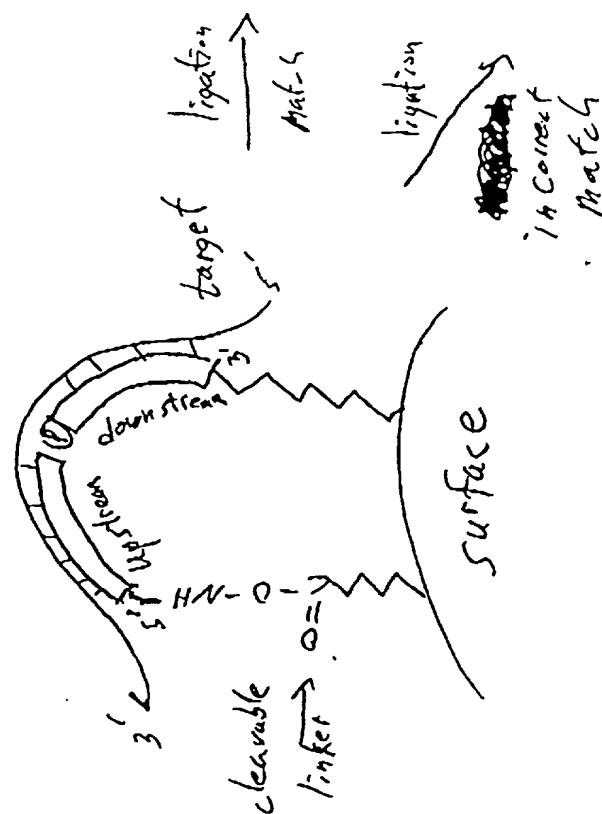


E

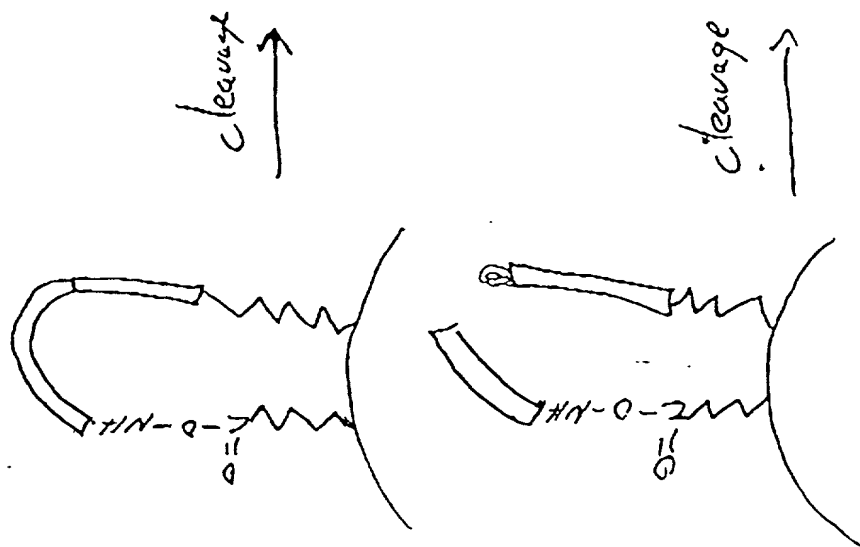
FIGURE 10  
(continued)

# SPOLA Assay

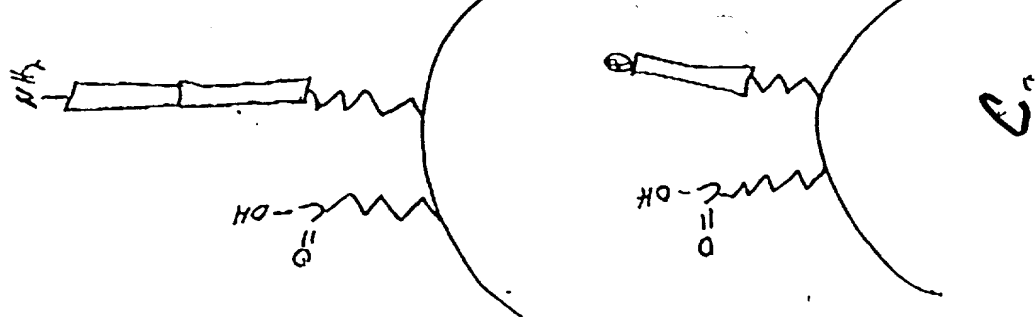
1521



A.



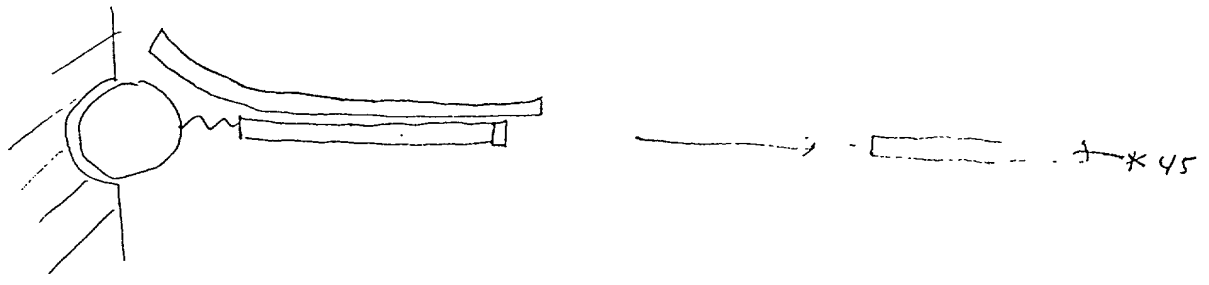
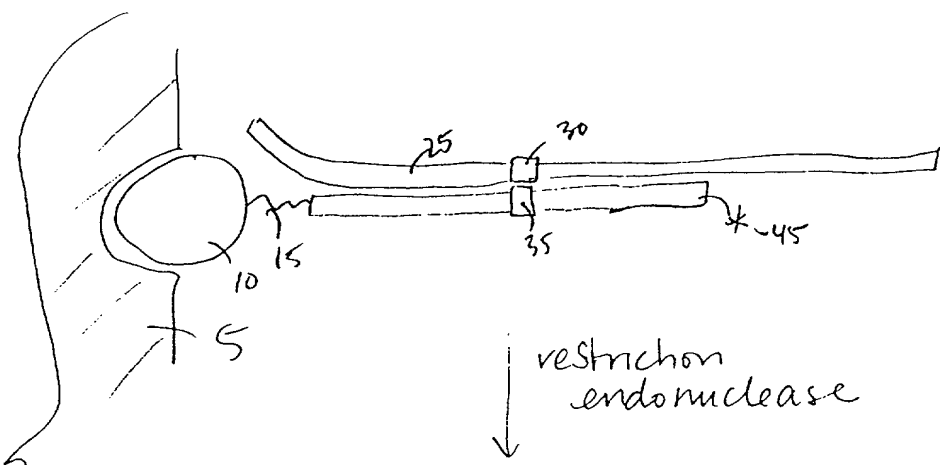
B.



C.

FIGURE 11

A



B

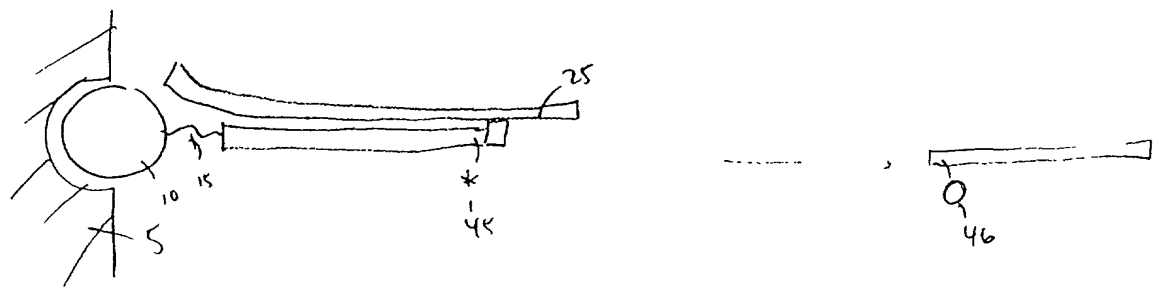
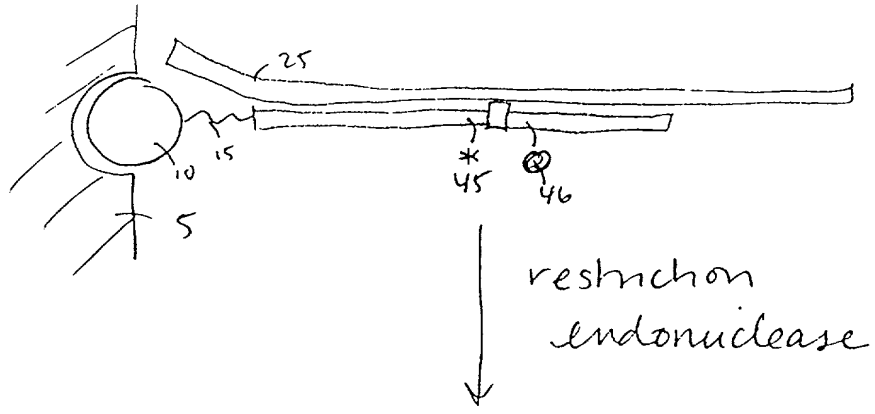
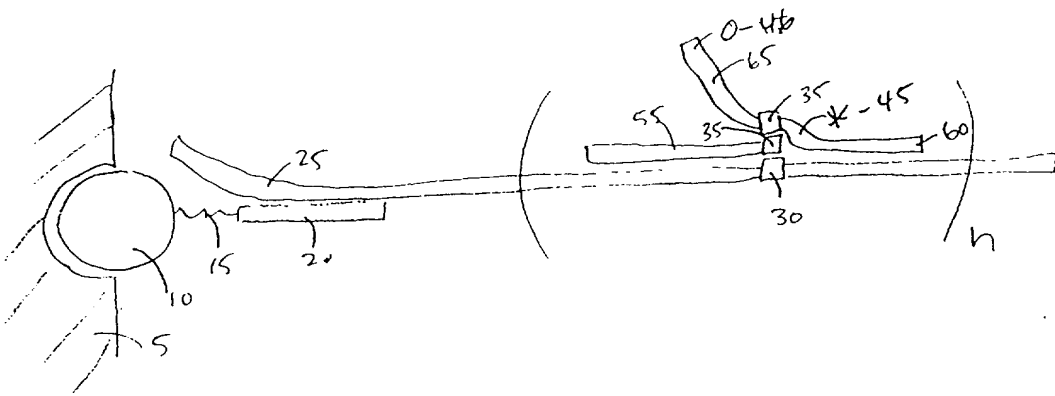
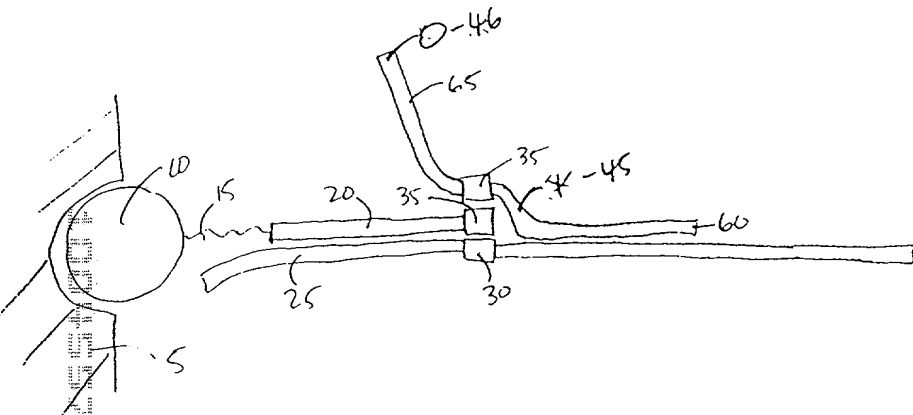


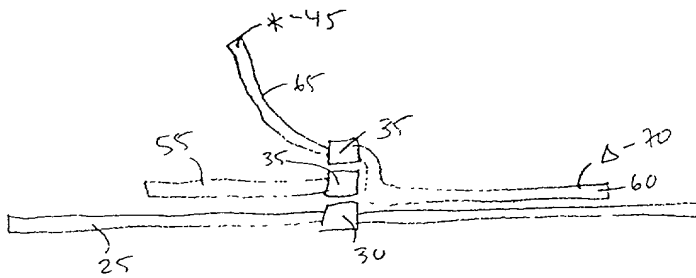
FIGURE 12



A

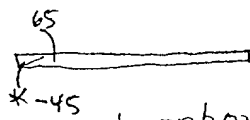


B



C

enzyme



optional remove undrained signal  
add to array probe,

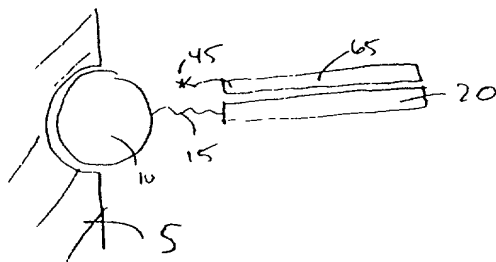
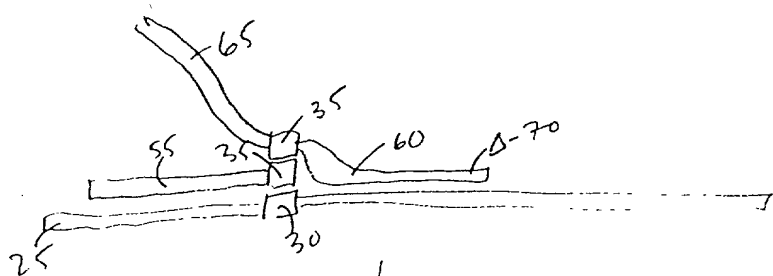
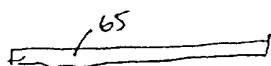


FIGURE 13



enzyme



optional removal of  
unreacted primers,  
optional ligase

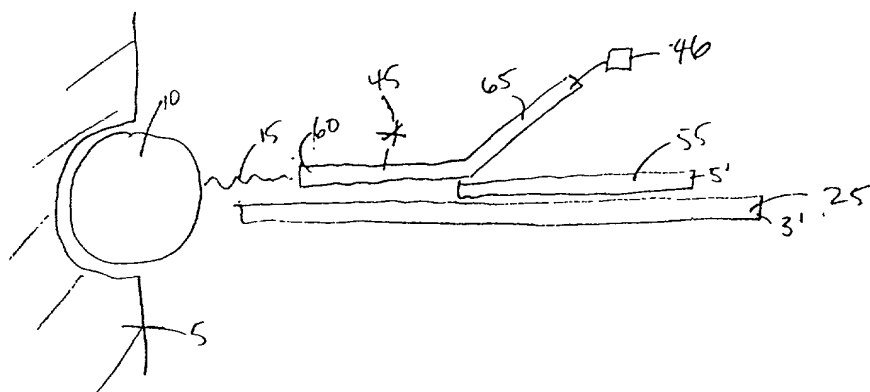
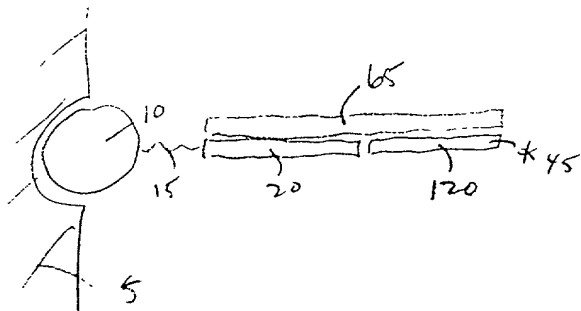
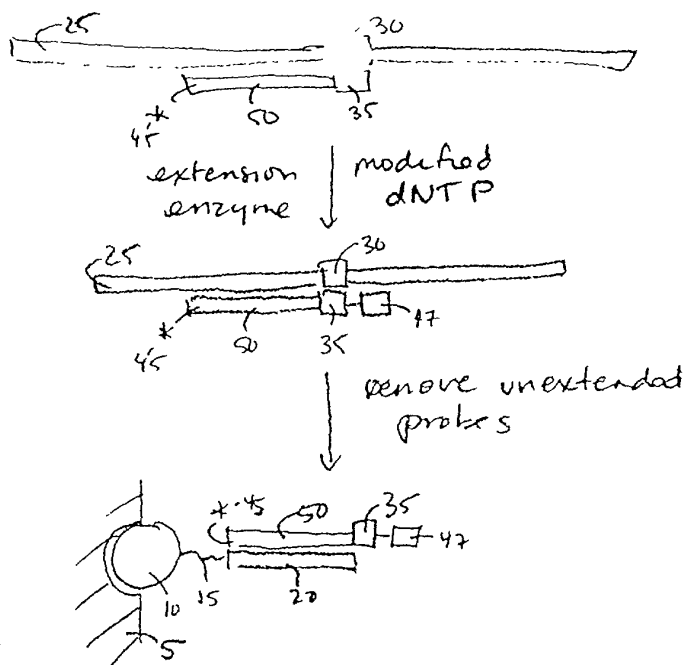
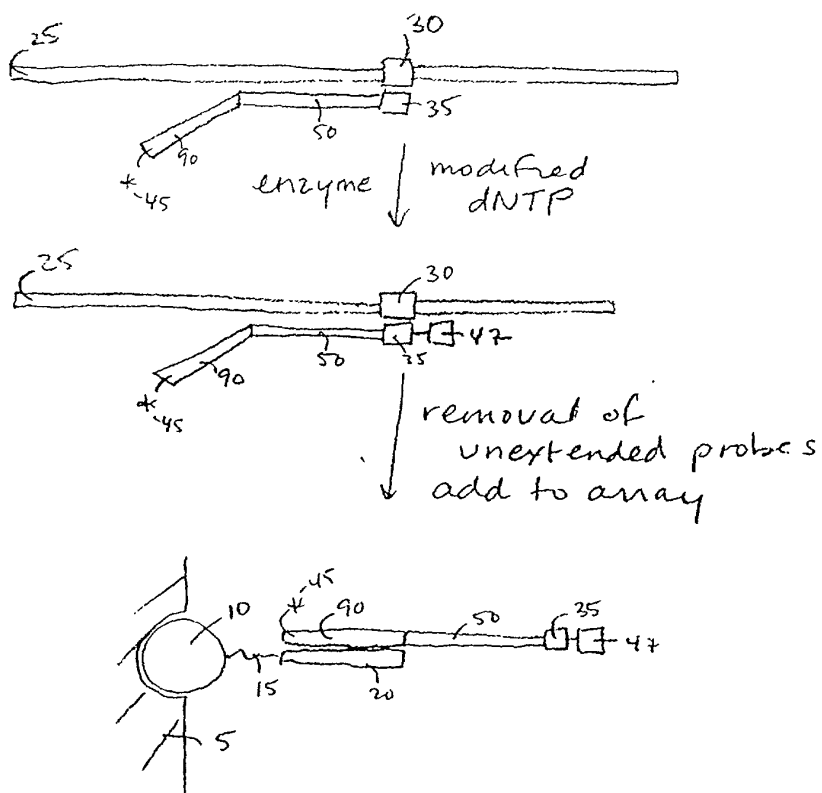


FIGURE 13  
(continued)



A



B

FIGURE 14

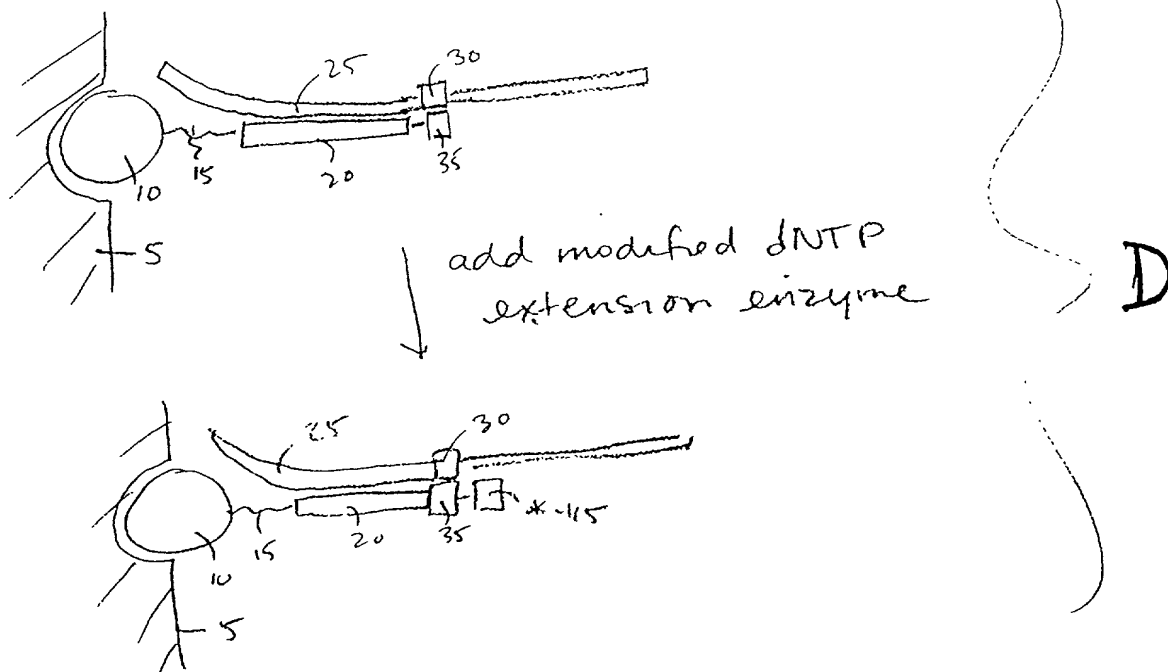
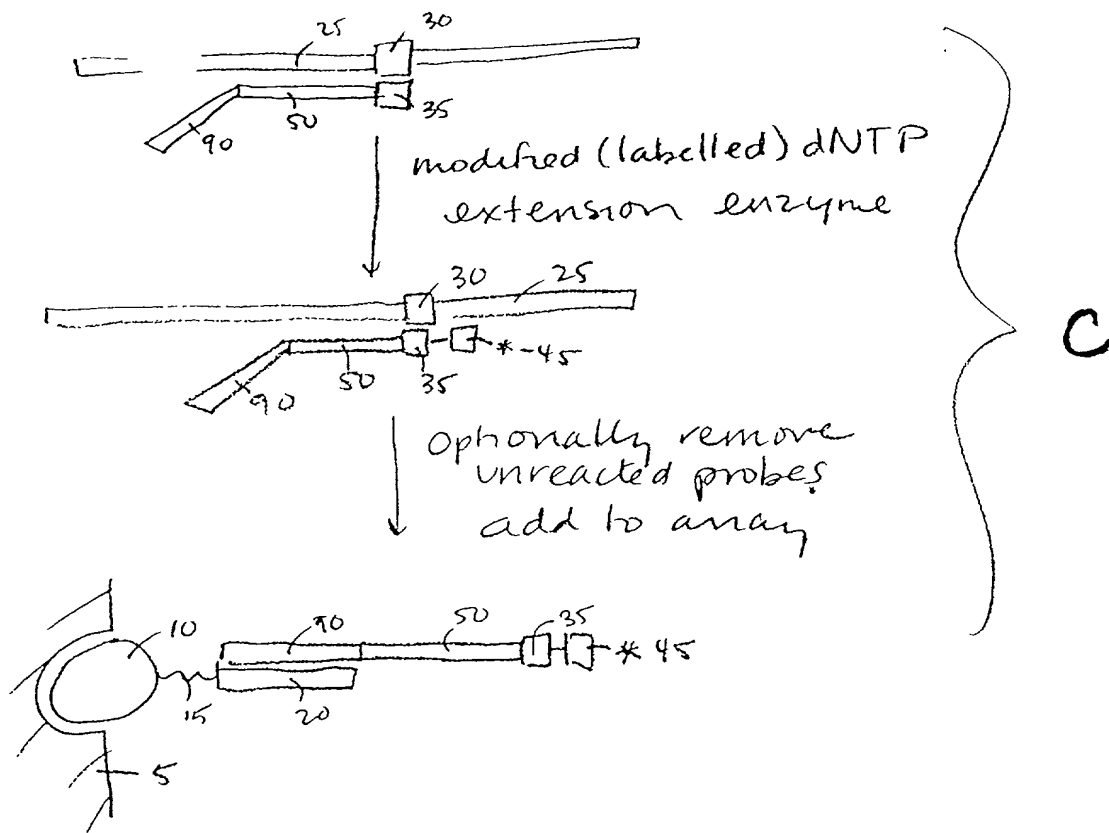


FIGURE 14 (continued)

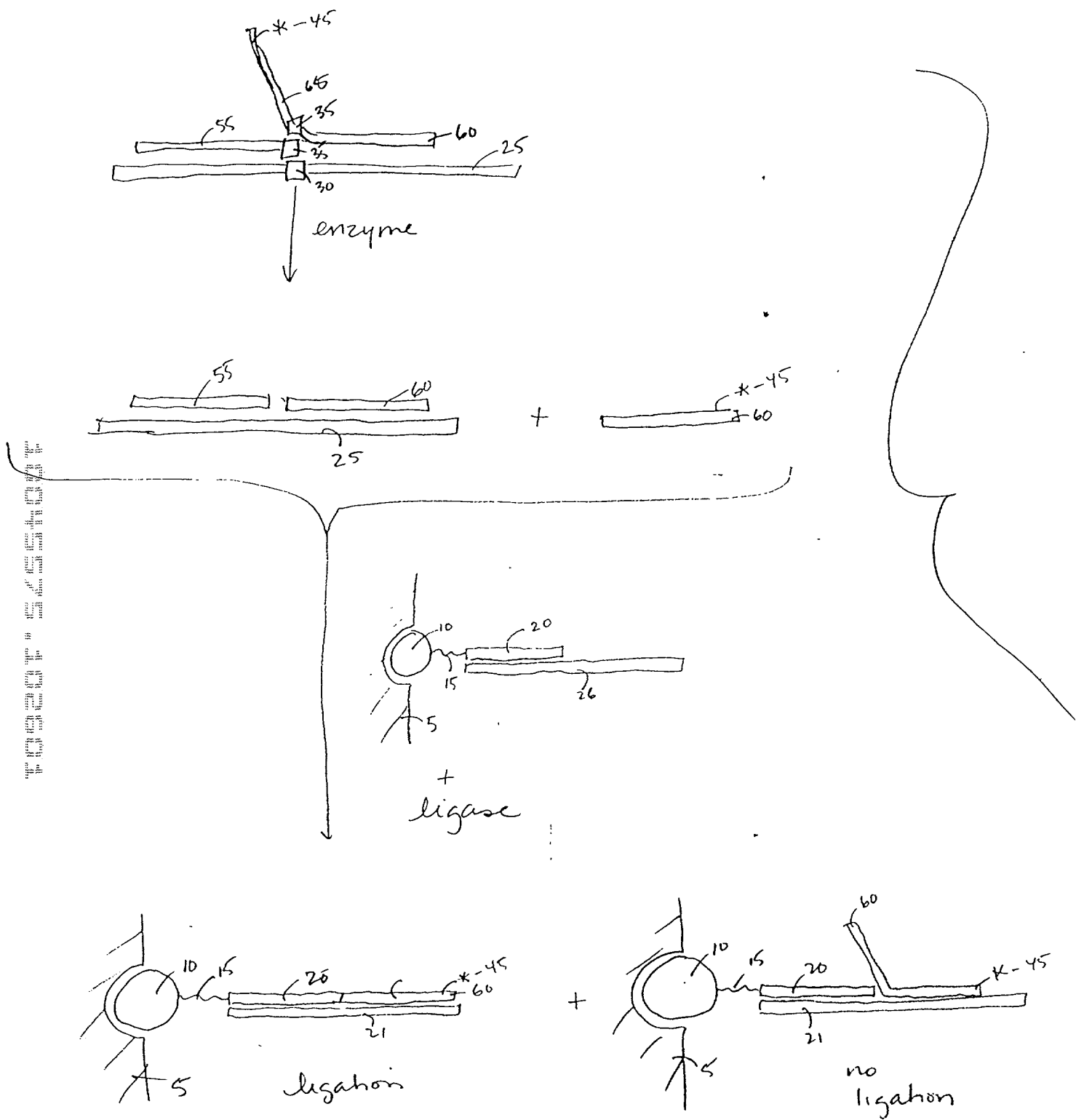
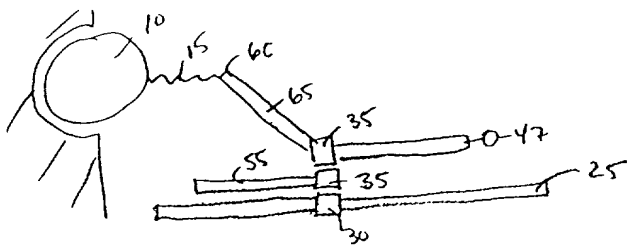
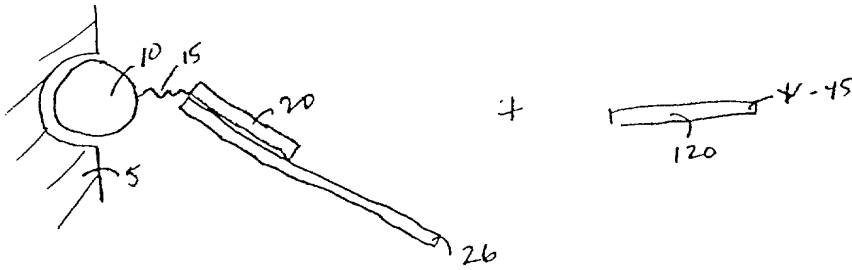


FIGURE 15A





↓ cleavage enzyme  
target template



↓ ligation

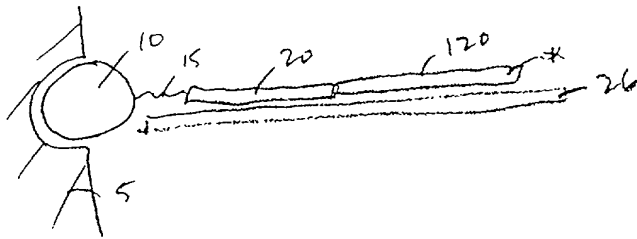


FIGURE 15B

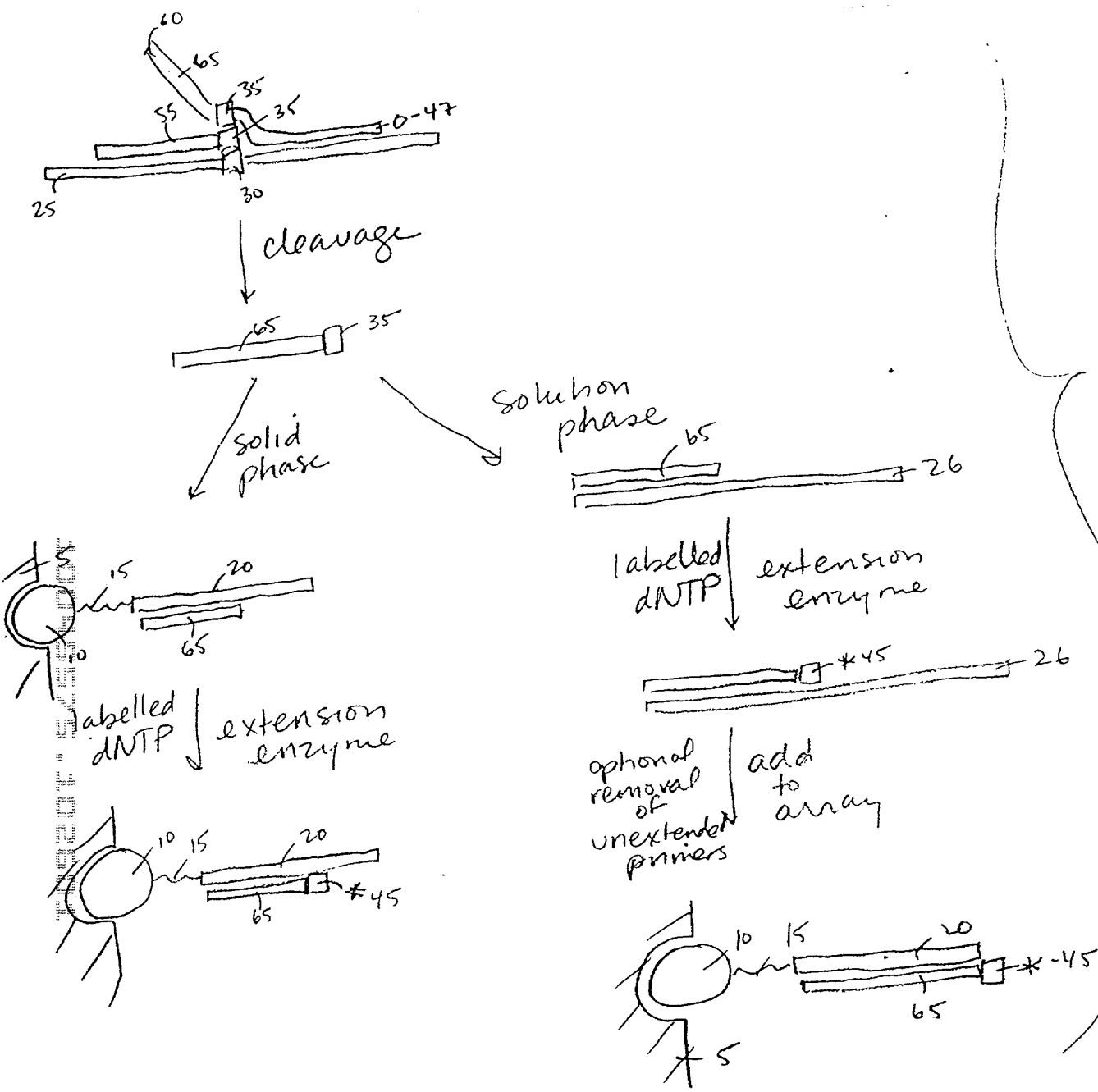


FIGURE 16A

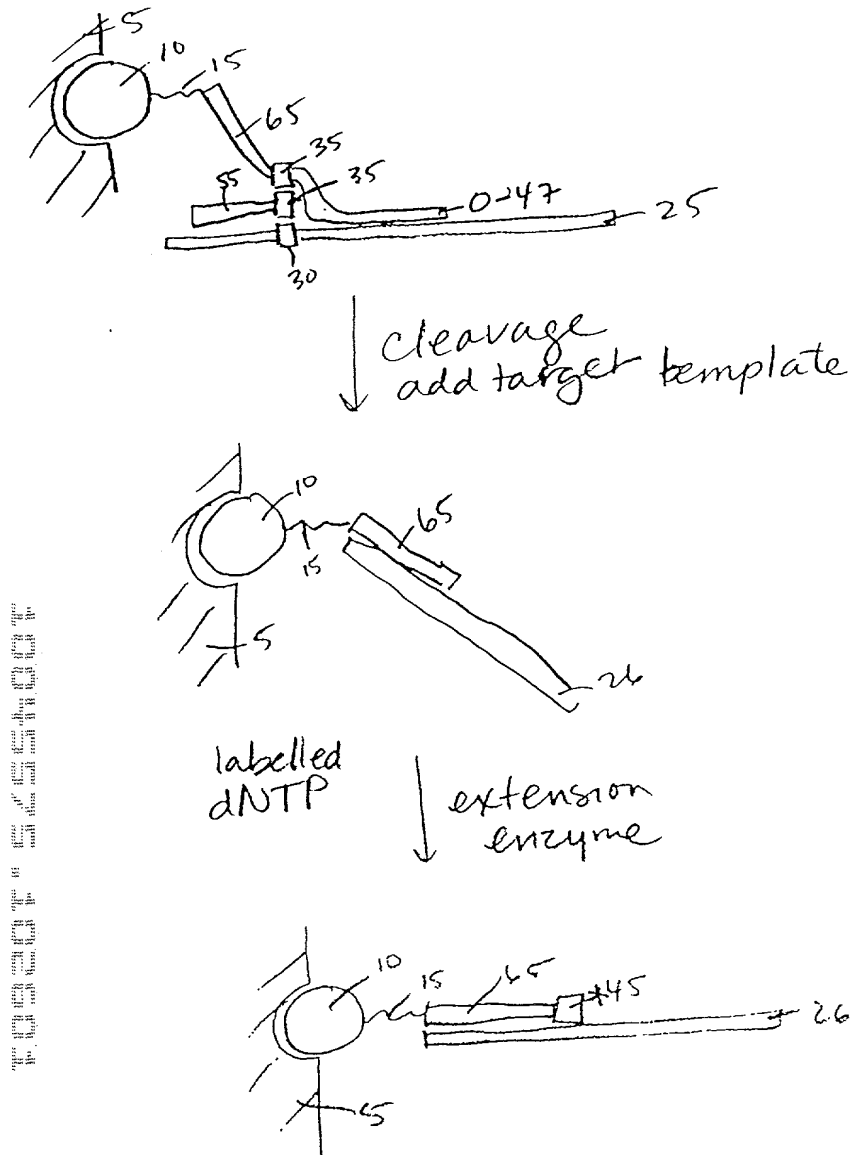


FIGURE 16 B

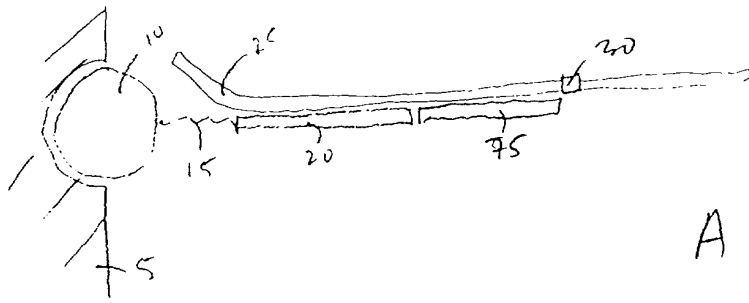
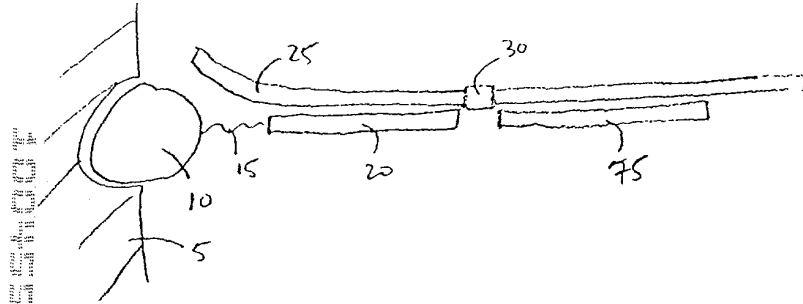
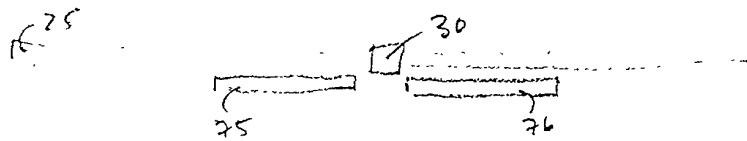


FIGURE 17

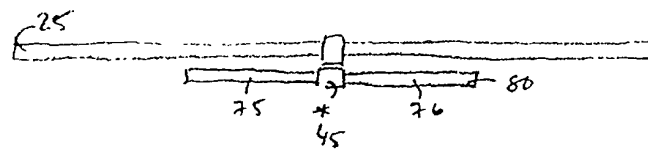
A



B

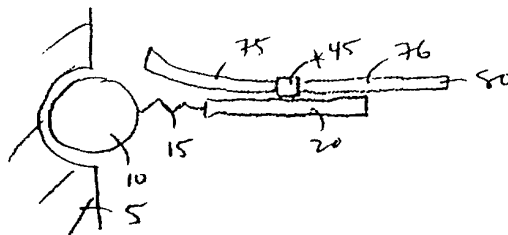


labeled dNTP  
extension enzyme  
ligase



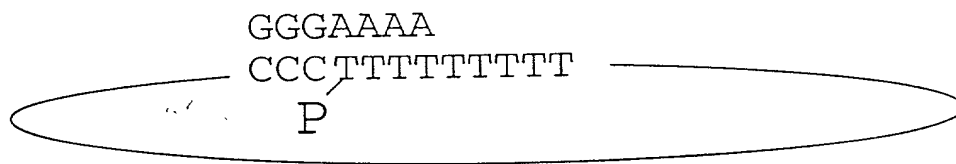
optional  
removal of  
unextended  
primers

denature  
add to array

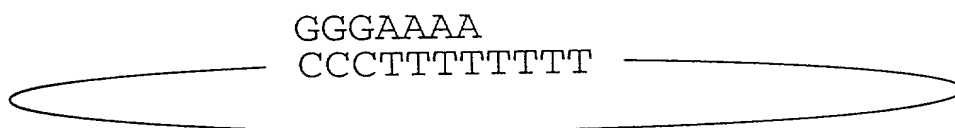


3'CCC ————— TTTTTTTT-P 5'  
cDNA

- (1) Circularize cDNA  
with guide linker



- (2) Ligate



- (3) Extend as  
Rolling circle

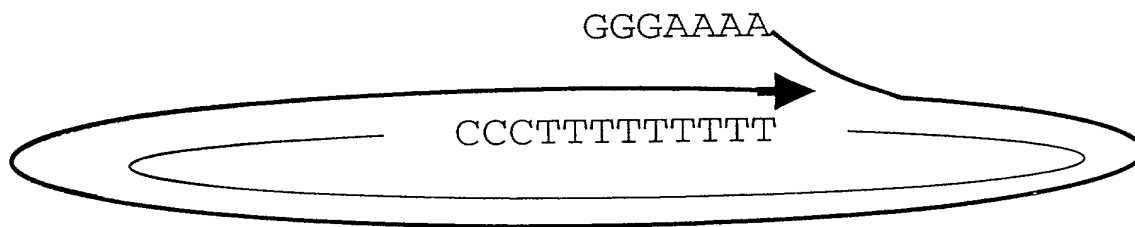


Figure 18

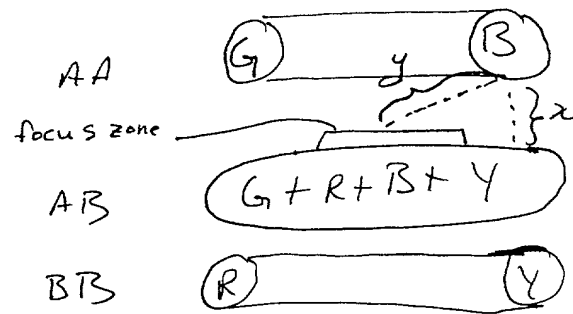
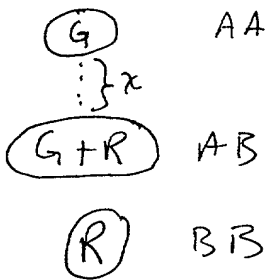
## Single Labeled Probe

Genotype	Signal
AA	G/G
AB	G/R
BB	R/R

## Multi-Labeled Probe

Genotype	Signal
AA	G <sub>1</sub> B / G <sub>2</sub> B
AB	G <sub>1</sub> B / R <sub>2</sub> Y
BB	R <sub>1</sub> Y / R <sub>2</sub> Y

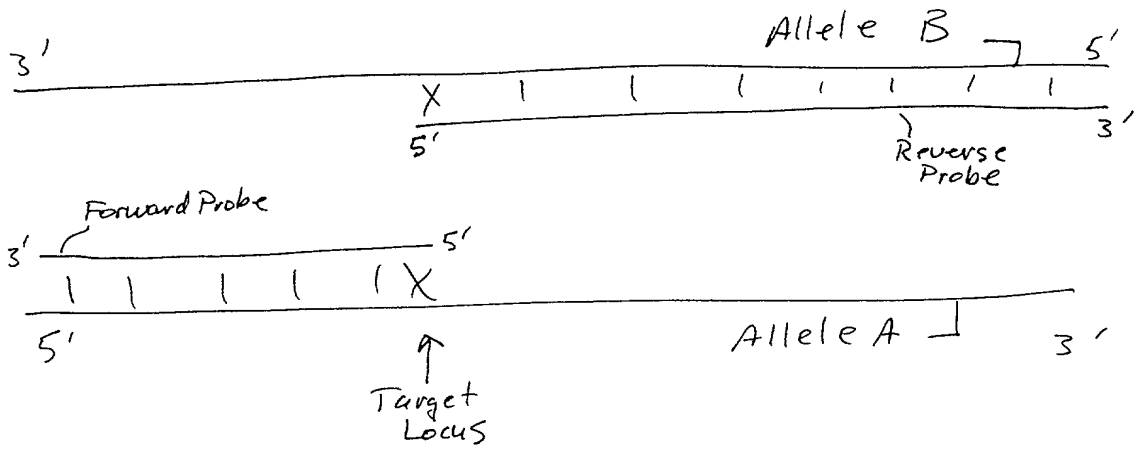
## Signal Range



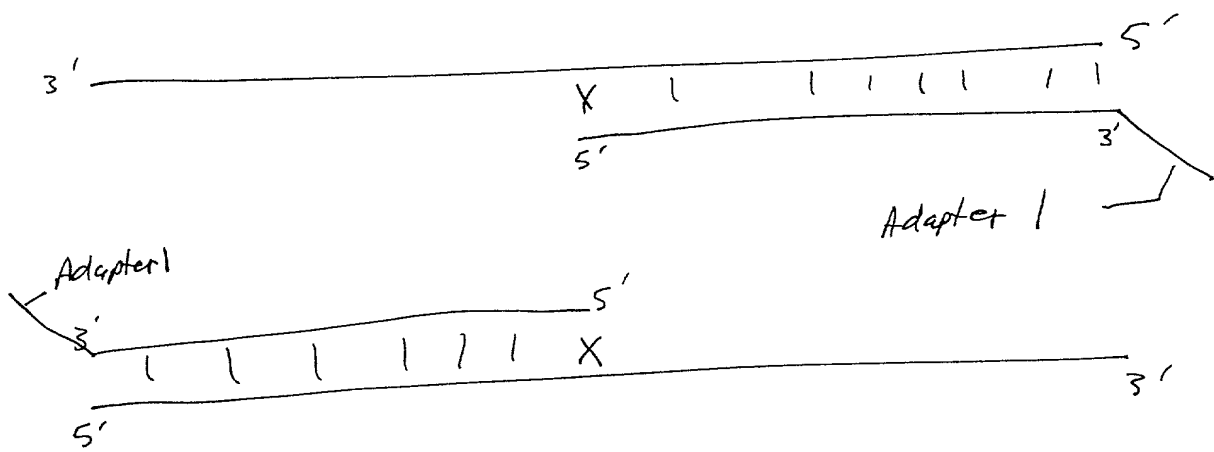
$x$  = single label distance  
 $y$  = multi label distance

Figure 19

A.



B.



C.

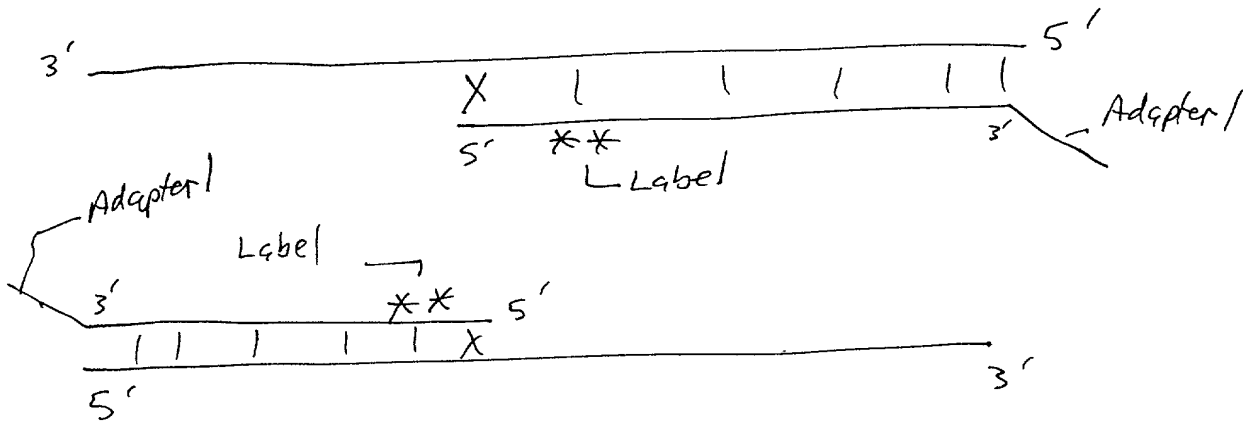


Figure 20

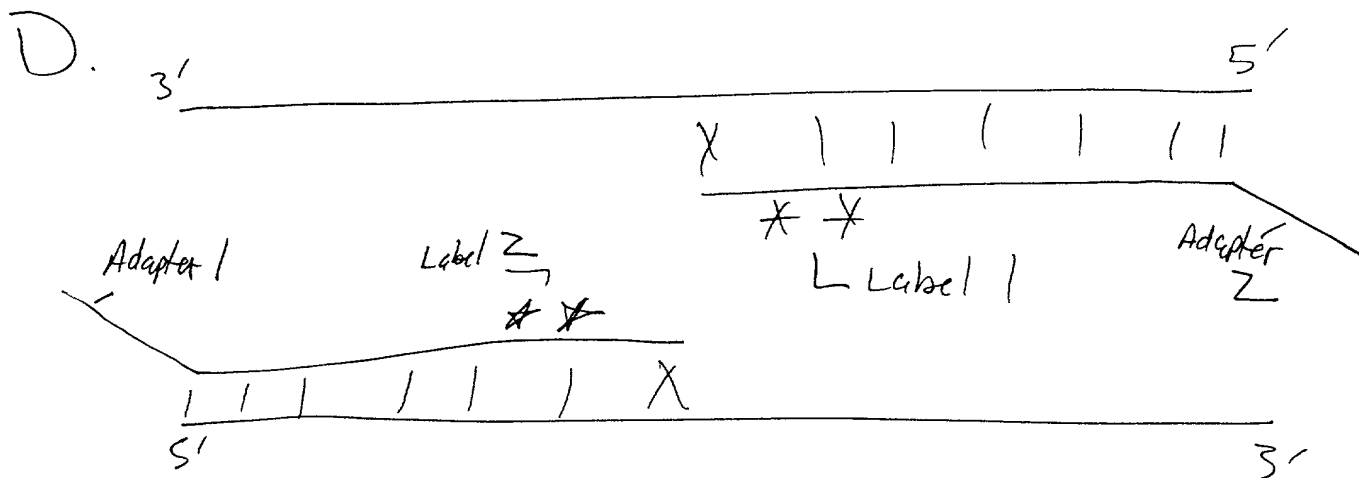


Figure 20 (continued)



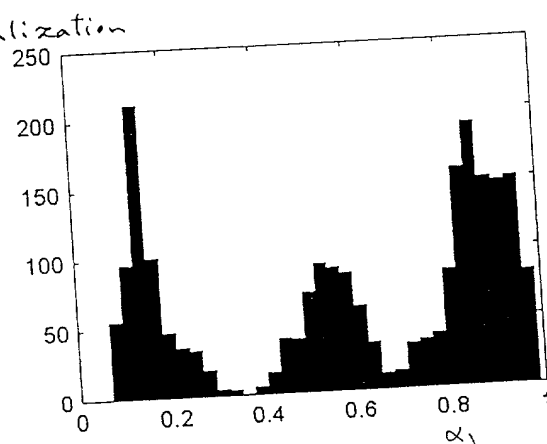
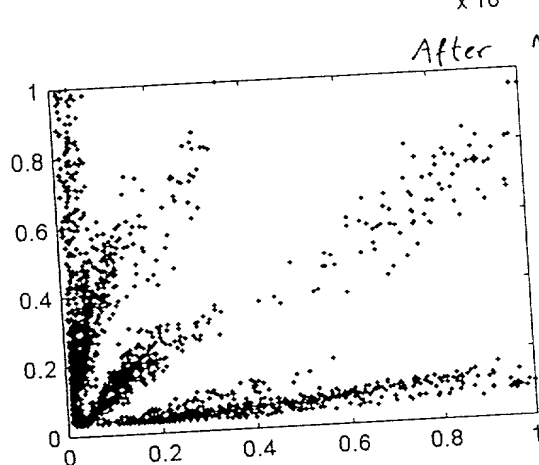
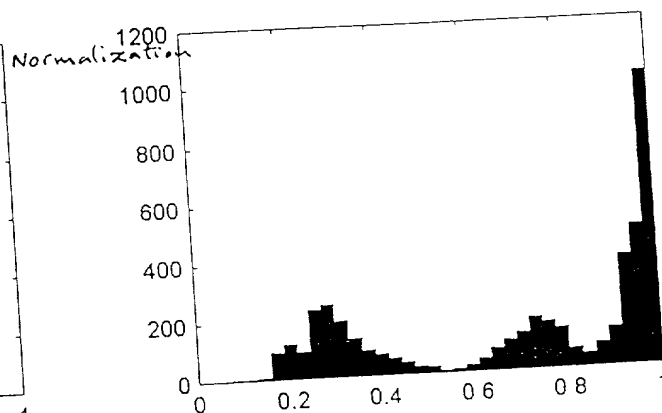
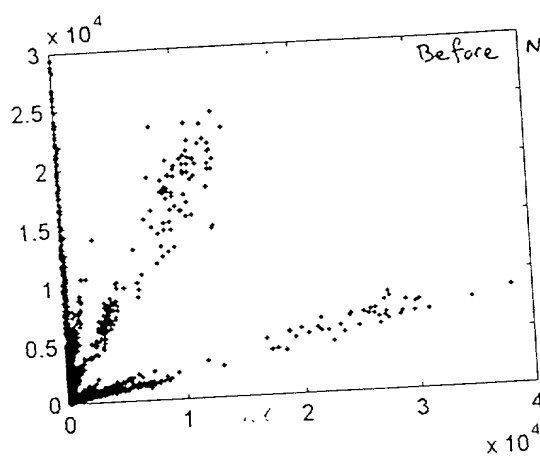
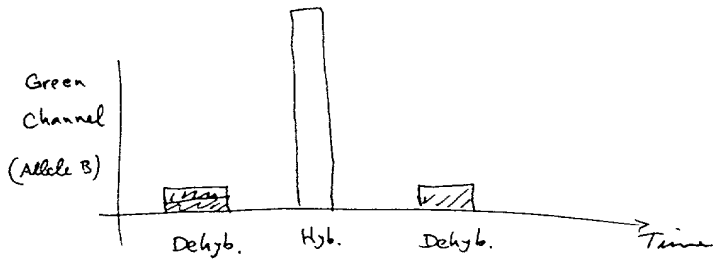
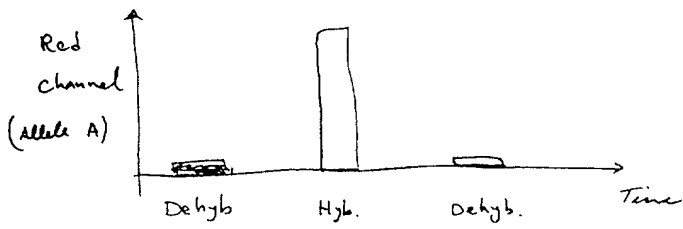


Figure 21



Figure

A

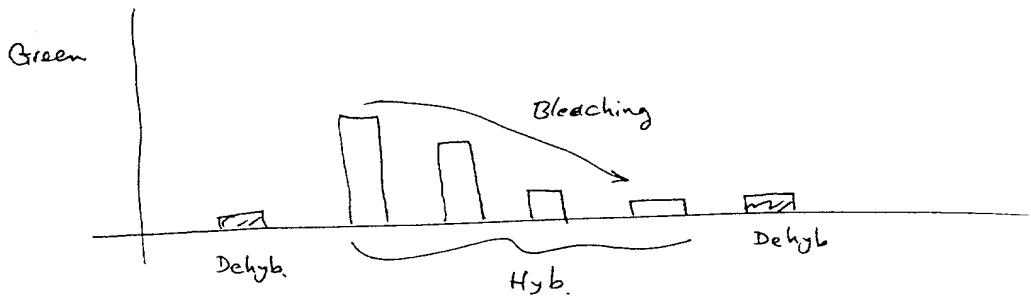
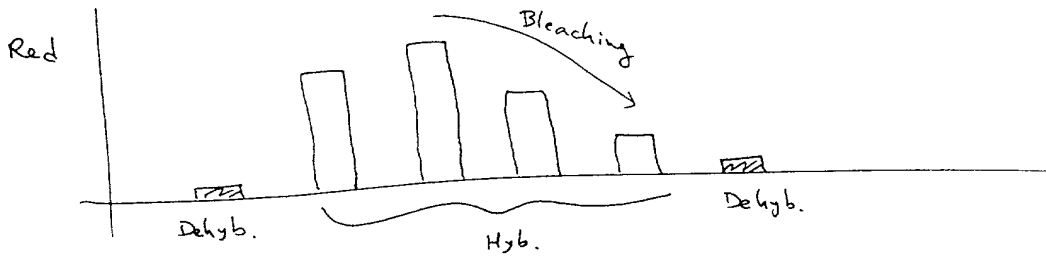


Fig. 22

B

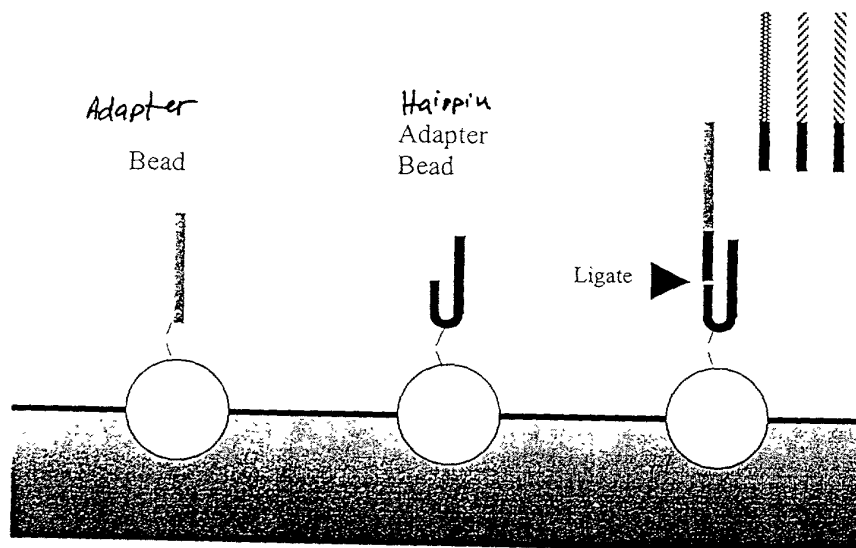
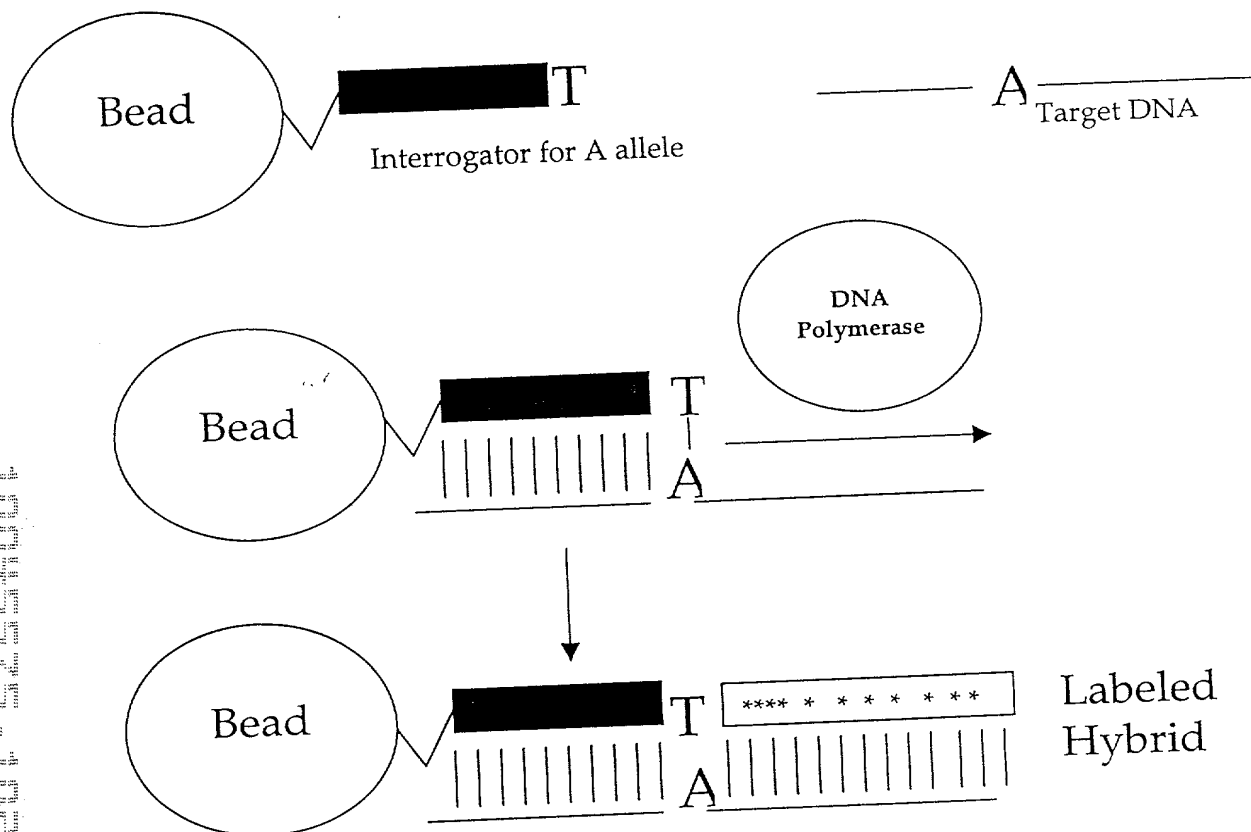


Figure 23

# A. Match to SNP allele



# B. Mismatch to SNP allele

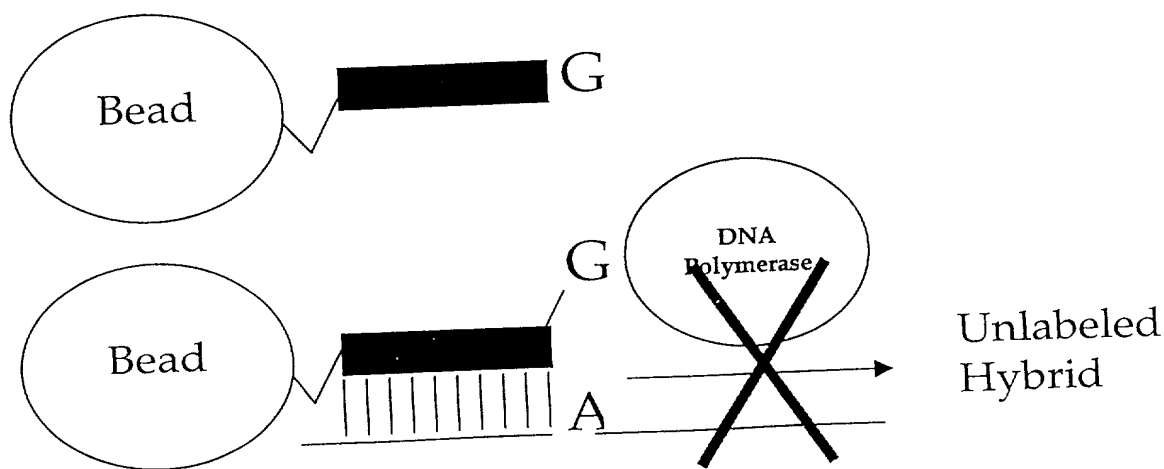
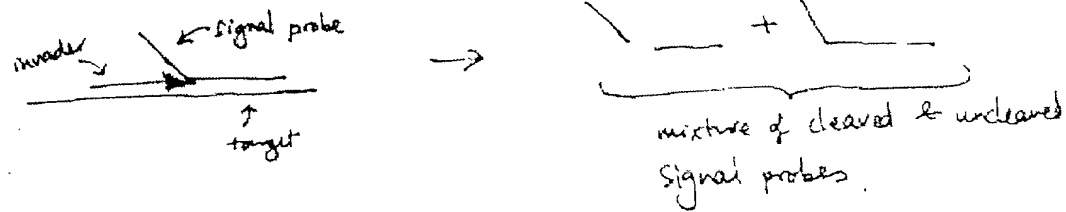


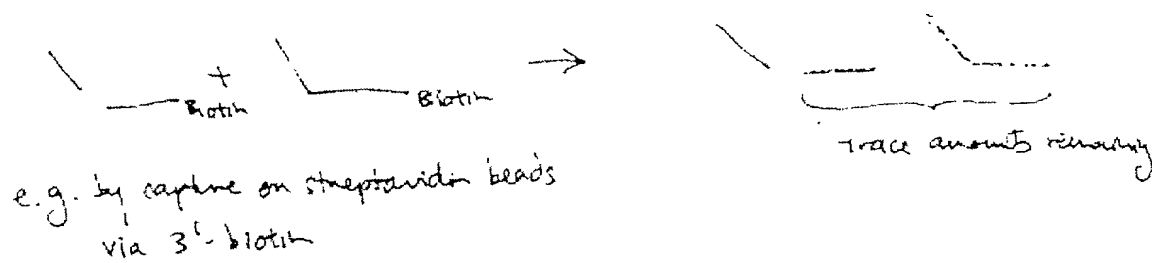
Figure 24

## Invader-PCR

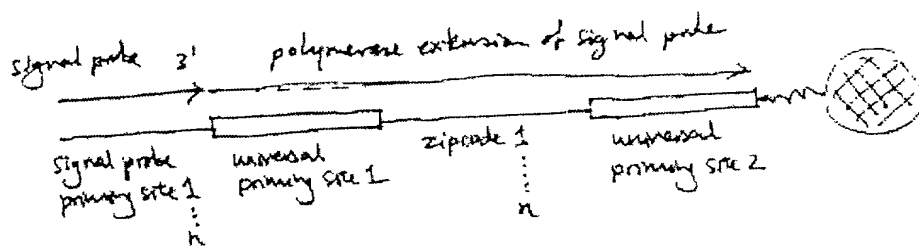
### 1) Invader reaction



### 2) Removal of uncleaved signal probes



### 3) Signal probe primes synthesis of amplicon target strand



### 4) PCR amplification

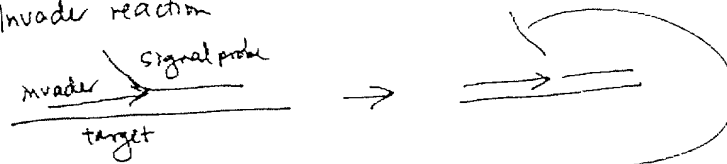
newly synthesized target strands are denatured from template & transferred to PCR reaction (universal primers, dNTPs, tag polymerase) for multiplex PCR. Universal primers are labelled e.g. with biotin.

### 5) Array hybridization - PCR amplicons containing zip codes are hybridized to array

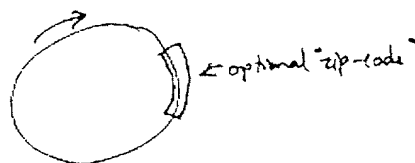
Figure 25

## Invader-Rolling Circle

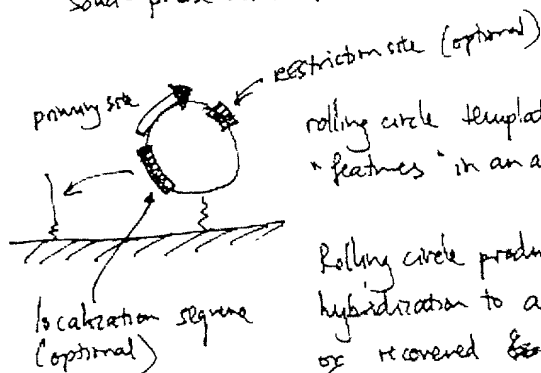
1) Invader reaction



Signal probe acts as primer on "rolling circle" template DNA



Solid-phase version:



rolling circle template is tethered to surface e.g. to localized "features" in an array format, or to beads.

Rolling circle products can be localized e.g. by hybridization to adjacent probes or recovered ~~in~~ in liquid phase for hybridization to a detection array. e.g. by enzymatic cleavage

Figure 26